Original Contribution

Journal of Cosmetic Dermatology, **0**, 1–6

Evaluation of anti-acne property of purified bee venom serum in humans

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Summary

Objective Acne vulgaris is a chronic dermatologic disease with four factors involved in the development of lesions. Treatments need to address as many of these underlying factors as possible in order to reduce acne lesions. As such, purified bee venom (PBV™) serum is an attractive therapeutic option for acne, but little data exist on the efficacy of this treatment strategy.

Methods In this prospective, noncomparative study, 30 subjects having mild-to-moderate acne vulgaris were enrolled and treated with PBV™ serum twice daily for a period of 6 weeks. Clinical evaluation of lesions by expert visual grading and image analysis were made at weeks 0 (baseline), 3, and 6.

Results The average visual acne grade of all volunteers significantly improved with the PBV™ serum treatment at weeks 3 ($P < 0.05$) and 6 ($P < 0.001$) when compared with the baseline grade at week 0. In addition, there was a mean percent improvement of 8.6% and 52.3% in acne grade observed after 3 and 6 weeks of PBV™ serum use, with 20% and 77% of the subjects showing improvement, respectively, when compared with baseline. Moreover, the subjects showed improvement in open comedones, closed comedones, papules, pustules, and nodules after 3 and 6 weeks of PBV™ serum use.

Conclusion Six weeks of treatment with PBV™ serum was found to be effective in the treatment of mild-to-moderate acne vulgaris, with no incidence of serious side effects or irritation.

Keywords: acne vulgaris, bee venom, *P. acnes*, lesion

Introduction

Multiple pathogenic factors are associated with acne vulgaris. As one of the most common dermatologic problems, acne involves the interplay of four main factors. They are excessive production of sebum, abnormal follicular keratinization, proliferation of anaerobic bacterium *Propionibacterium acnes*, and the release of inflammatory mediators.[1] According to the current concept of acne pathogenesis, sebaceous hyperplasia and follicular hyperkeratinization under the influence of androgens cause a change in follicular milieu with consecutive proliferation of bacteria. This leads to increased production of pro-inflammatory cytokines which stimulate comedogenesis at the level of follicular keratinocytes. Further inflammatory responses via the activation of cell-mediated immune processes

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Accepted for publication April 5, 2016

lead to the increasing severity of acne.[2] Interestingly, the association between dietary intake and the pathogenesis of acne still remains slim and debateable although in recent times the hypothesis of the role of nutrition provides a rational basis in the development of acne.[3]

P. acnes contributes to the inflammatory reaction of acne by inducing monocytes and keratinocytes to produce pro-inflammatory cytokines, including IL-1β, IL-8, and TNF-α.[4] The induction of these cytokines by *P. acnes* is mediated by Toll-like receptor (TLR) 2.[5] Various therapeutic agents including antibiotics for acne have been used to inhibit inflammation or kill bacteria. However, antibiotics may lead to the emergence of resistant pathogens and side effects. Thus, a group of researcher recently studied the anti-inflammatory property of bee venom that includes heat-killed *P. acnes* on human keratinocyte and monocyte cell lines. Kim et al[6] investigated the anti-inflammatory effects of bee venom in heat-killed *P. acnes*-treated HaCaT and THP-1 cells, as revealed by ELISA analysis and Western blotting by measuring the pro-inflammatory cytokines and chemokines. Heat-killed *P. acnes* markedly increased the secretion of TNF-α, IL-8, and IFN-γ in HaCaT and THP-1 cells. However, bee venom treatment decreased the secretion of those cytokines. In addition, bee venom inhibited heat-killed *P. acnes*-induced TLR2 expression in HaCaT cells. These results suggested that bee venom blocked TLR2 expression and suppressed the production of pro-inflammatory cytokines induced by *P. acnes* in HaCaT and THP-1 cells. Another recent study[7] reported that bee venom has a potential antibacterial effect against inflammatory skin disease. In this context, *P. acnes* was intradermally injected into ears of ICR mice. Following the injection, bee venom mixed with Vaseline was applied to the skin surface of the ear. Histological observation revealed that the *P. acnes* injection induced a considerable increase in the number of infiltrated inflammatory cells and inflammatory cytokines. By contrast, the bee venom-treated ears showed noticeably reduced ear thickness. Additionally, bee venom significantly inhibited the number of TNF-α- and IL-1β-positive cells.

Purified bee venom (PBV™) was developed for reducing the acne formation associated with inflammatory process. However, there has been little research into anti-acne effects of PBV™ containing serum in humans. Therefore, the aim of the present study was to determine whether the use of an anti-acne product reduced the appearance of acne on the facial skin.

Materials and methods

Purified honeybee venom collection

Purified bee venom (PBV™) added into the serum was from colonies of natural honey bees (*Apis mellifera* L.). Bee venom was collected with a bee venom collector (Chungjin Biotech, Korea) in a sterile manner and purified under strict laboratory conditions. In brief, the collected venom was diluted in cold sterile water and then centrifuged at 10 000 *g* for 5 min at 4 °C to discard residues from the supernatant. Purified bee venom (PBV™) was lyophilized by freeze dryer and refrigerated at 4 °C for later use.

Subjects

The clinical study was conducted in accordance with the intent and purpose of Good Clinical Practice regulations described in Title 21 of the US Code of Federal Regulations, the Declaration of Helsinki and/or Essex Testing Clinic Standard Operating Procedures. A total of 30 participants (77% Caucasian) aged 12–33 years, diagnosed as having mild-to-moderate acne vulgaris, and attending the Essex Te sting Clinic, Inc. (Verona, NJ, USA), were studied. All subjects had no prior experience with the skin care regimen. The assessment of acne was graded by the board-certified dermatologist according to the modified Cook's scale (Table 1).[8] To be enrolled, the baseline score had to be a "2" or "3". The dermatologist also identified and counted the number of acne lesions on the cheeks, chin, and forehead. All procedures involved in the study were explained in detail to volunteers, and written informed consent was obtained from each subject prior to entering the study. A copy of the informed consent was provided to each subject. Subjects with prescription treatment for acne, a propensity for pregnancy, use of sensitive cosmetics or personal care products, a sensitivity or allergy to bees, honey, avocado oil, olive oil, shea butter, coconut, vitamin E, or listed ingredients of the test product were excluded. Subjects meeting the inclusion/exclusion criteria were empaneled. Enrolled subjects had digital photographs taken and they were instructed to apply PBV™ serum on their entire facial skin with an amount of 0.7–0.9 g twice daily in the morning and evening after washing face with the supplied foaming facial cleanser for 6 weeks. Clinical evaluations were made at weeks 0 (baseline), 3 and 6. Dermatologist's visual assessment and photographs were used to evaluate changes in acne lesions.

Table 1 Modified Cook's Acne Grading Scale*

Grade	Description
0	Facial skin needs not be perfectly clear. A few scattered comedones or papules may be present, but these should be visible only on close examination
1	Comedones and small papules are present and noticeable from a distance of 1–3 feet away
2	About one-fourth (25%) of facial area is involved, with small papules (about 6–12) and comedones (a few pustules or large prominent papules may be present)
3	Approximately 30% (26–49%) of facial area is involved, with small papules (13–20) and small comedones (a few pustules or large prominent papules may be present)
4	Approximately half (50%) of facial area is involved, with small papules and large or small comedones. A few pustules or large prominent papules are usually present. (If lesions are generally large, subject may have a grade "4" severity, although less than half of facial area is involved.)
5	More than half (51–74%) of facial area is involved with large and small papules and comedones (Less facial area of involvement is permissible if inflammatory lesions are large). A moderate number of pustules are usually present, some of which may be large
6	Approximately three fourths (75%) of facial area is involved, with papules and/or large open comedones. (Less facial area of involvement is permissible if inflammatory lesions are large). Numerous pustules are usually present, some of which may be large
7	Greater than 75% but less than 85% of facial area is involved with lesions with the majority being papules and large open comedones. Pustules may be large and prominent

*Cook *et al.*, 1979[8].

Visual assessment of subjects

For a couple of hours preceding the clinic visit at weeks 0, 3, and 6, subjects were instructed not to make any changes to their daily skin care routine or expose their face to intense sunlight. Upon arrival, subjects waited at constant temperature (22 ± 2 °C) and humidity (50 ± 5%) before assessment by dermatologist. The subjects were evaluated for acne lesions based on a lesion scale (Table 2). At each visit, additional scale was used to assess irritation on the face of each subject (Table 3). This evaluation was for safety purpose only and was not used in determining efficacy of bee venom serum.

Table 2 Assessment scale for acne lesions

Scale	Description
1	Open comedones = blackheads
2	Closed comedones = whiteheads
3	Papules = raised circular areas without pus
4	Pustules = small, inflammatory lesions with pus
5	Nodules = large inflamed lesions

Table 3 Assessment scale for facial irritation

Scale	Description
0	No evidence of any irritation
+	Barely perceptible irritation present
1	Mild irritation present
2	Moderate irritation present
3	Marked irritation present
4	Severe irritation present

Image analysis

At all visits, digital images of the face of each subject were taken using the Janus Digital Imaging System. Photographs were taken from the front, right, and left views. To ensure consistency between the photographs, each subject was draped with a black cloth around the shoulders to eliminate the appearance of clothing in the pictures and each subject wore a black headband to pull hair off of and away from the face. The images were analyzed using Image Pro software (Media Cybernetics, Bethesda, MD, USA) to determine changes in the acne lesions.

Statistical analysis

The data were presented as the mean ± standard deviation (SD). The changes from the baseline of efficacy parameters were evaluated. The paired *t*-test was used to determine the statistical significance of differences in efficacy of PBV™ serum. The data analysis was performed using SPSS software version 20.0 (SPSS Inc., Chicago, IL, USA).

Results

As shown in Table 4 and Fig. 1, the average visual acne grade of all volunteers significantly improved with PBV™ serum treatment at weeks 3 (1.9 ± 0.6, $P = 0.031$) and 6 (1.0 ± 0.8, $P < 0.001$) when compared with the baseline grade at week 0 (2.1 ± 0.6). Furthermore, there was a mean percent improvement of 8.6% and 52.3% in acne grade observed after 3 and 6 weeks of PBV™ serum use, with 20% and 77% of the subjects showing improvement, respectively, when compared with baseline. When visual acne lesion counts were compared with baseline (Table 5), there was a decrease in open comedones after 3 and 6 weeks of PBV™ serum use, with the greatest improvement observed after

Table 4 Changes in the visual acne grade in patients treated with purified bee venom (PBV™) serum

Week	Grade[1]	Mean % change from baseline	% of subjects with improvement from baseline
0	2.1 ± 0.6	–	
3	1.9 ± 0.6*	−8.6	20
6	1.0 ± 0.8**	−52.3	77

[1]Mean ± SD.
*$P < 0.05$, **$P < 0.001$ compared to baseline at week 0.

6 weeks of PBV™ serum use, and it was statistically significant ($P < 0.001$) when compared with baseline. Likewise, there was a significant decrease ($P < 0.001$) in closed comedones after 6 weeks of PBV™ serum use when compared with baseline. Interestingly, there was a decrease in papules after 3 and 6 weeks of PBV™ serum treatment which were statistically significant ($P < 0.05$) when compared with baseline. Analysis on pustules and nodules revealed that there was no change observed after 3 and 6 weeks of PBV™ serum use. On the other hand, the images of acne lesion were analyzed to determine changes in lesion counts from PBV™ serum use (Table 6). There was a decrease in open and closed comedones after 3 and 6 weeks of PBV™ serum use, both of which were statistically significant ($P < 0.001$) when compared with baseline. Contrary to the visual acne lesion counts, there was a slight decrease in

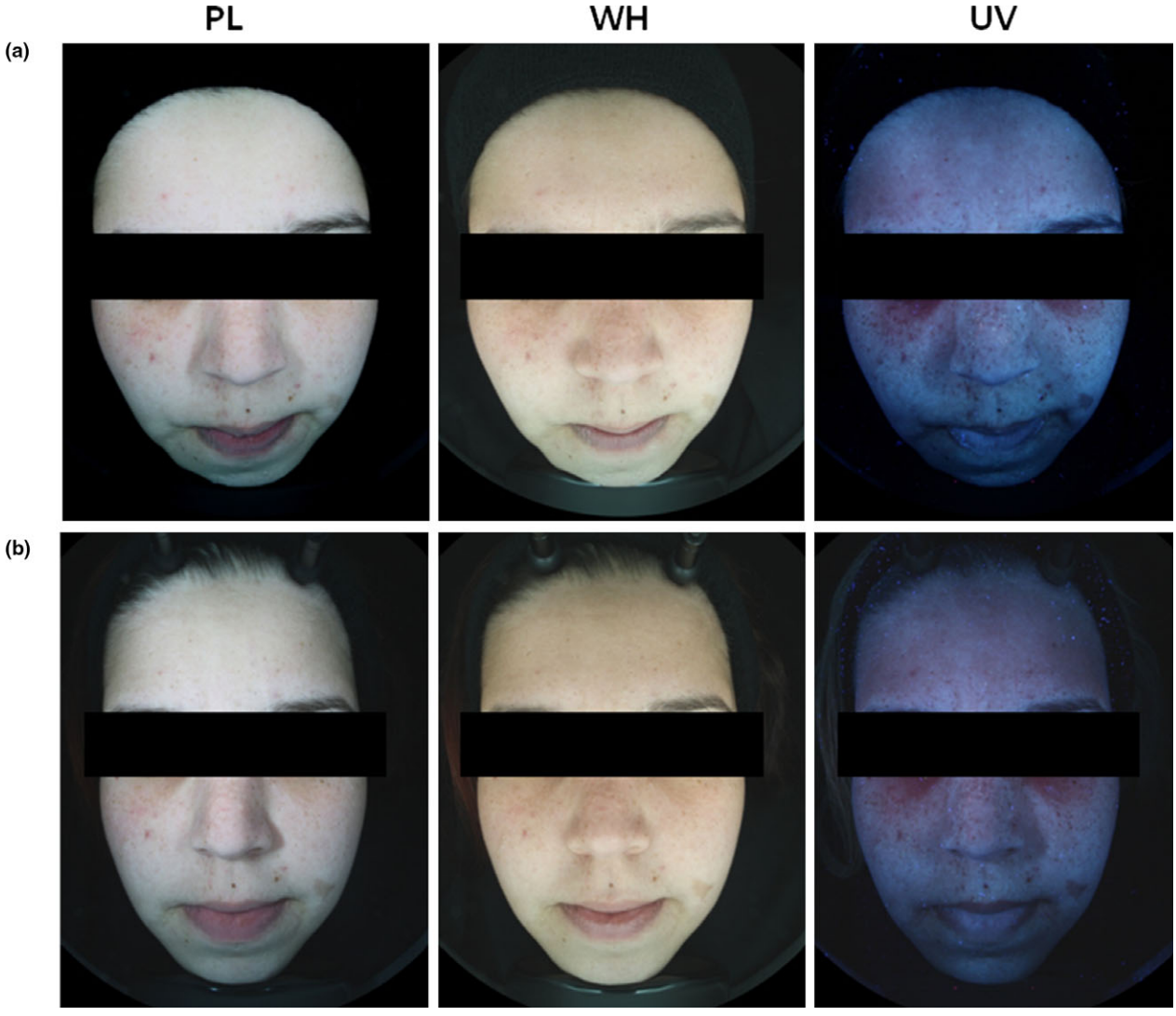

Figure 1 Baseline (a) and after the six-week treatment with Purified bee venom (PBV™) serum (b). Three representative patients with grade 3 (a) acne scars were improved to grade 1(b). PL, Photoluminescence photography; WH, using white light lamp; UV, UV photography.

Table 5 Changes in the visual acne lesion counts* in patients treated with purified bee venom (PBV™) serum

Week	Open comedones	Closed comedones	Papules	Pustules	Nodules
0	2.5 ± 2.6	3.6 ± 2.6	5.3 ± 2.8	0.2 ± 0.5	0.0 ± 0.0
3	2.2 ± 2.4 (−12.0%)	4.2 ± 2.9 (+16.7%)	2.7 ± 2.2 (−49.1%)	0.0 ± 0.2 (−100.0%)	0.0 ± 0.0 (0.0%)
P value	0.227	0.202	<0.001	0.063	1.000
6	0.2 ± 1.1 (−92.0%)	1.0 ± 1.6 (−72.2%)	3.0 ± 2.4 (−43.4%)	0.2 ± 0.6 (0.0%)	0.0 ± 0.2 (0.0%)
P value	<0.001	<0.001	<0.001	0.594	1.000

*Mean ± SD.

Table 6 Changes in the acne lesion counts by image analysis* in patients treated with purified bee venom (PBV™) serum

Week	Open comedones	Closed comedones	Papules	Pustules	Nodules
0	16.8 ± 19.5	99.7 ± 112.5	2.6 ± 1.8	2.1 ± 2.2	1.0 ± 1.2
3	6.9 ± 8.4 (−58.9%)	69.8 ± 74.0 (−30.0%)	2.5 ± 1.7 (−3.8%)	2.0 ± 3.2 (−4.8%)	0.6 ± 1.0 (−40.0%)
P value	<0.001	0.004	0.472	0.773	0.177
6	5.8 ± 6.3 (−65.5%)	53.5 ± 60.9 (−46.3%)	2.9 ± 2.9 (+11.5%)	1.9 ± 2.4 (−9.5%)	0.8 ± 1.0 (−20.0%)
P value	<0.001	<0.001	0.722	0.482	0.292

*Mean ± SD.

papules after 3 weeks of PBV™ use and an increase in papules after 6 weeks of PBV™ serum use, neither of which was statistically significant when compared with baseline. There was a decrease in pustules and nodules after 3 and 6 weeks of PBV™ serum use, neither of which was statistically significant when compared with baseline with the greatest improvement observed in pustules and nodules after 6 and 3 weeks of PBV™ serum use, respectively. There was no irritation observed on any subject during the course of the study.

Discussion

The main aim of the present study was to evaluate the clinical effects of PBV™ serum by counting the acne lesions objectively using visual evaluation and image analysis. Our research demonstrated that PBV™ serum treatment clinically improved the appearance of acne on the face by decreasing mean acne grade. Moreover, the subjects showed improvement in open comedones, closed comedones, papules, pustules, and nodules after 3 and 6 weeks of PBV™ serum use.

During an inflammatory response, TLR activation results in the mobilization of the MAPK and the transcription factor NF-κB signaling pathways. These pathways then modulate inflammatory gene expression, which is crucial in shaping the innate immune response within the inflammatory skin disease.[9] Lee et al[10] investigated the effects of melittin in the production of inflammatory cytokines in heat-killed *P. acnes*-treated HaCaT cells. Administration of heat-killed *P. acnes* increased expression of IKK, IκB, and NF-κB in HaCaT cells. However, the addition of melittin reduced IKK, IκB, and NF-κB phosphorylation. It indicates that treatment with melittin abrogated the effect of *P. acnes* in altering the expression through NF-κB signaling. The same study investigated whether melittin modulates MAPK signaling in heat-killed *P. acnes*-treated HaCaT cells. Findings showed that phosphorylated p38 was markedly increased after treatment with heat-killed *P. acnes;* however, phosphorylated p38 was decreased after treatment with melittin. These results underscore the theory that melittin inhibits pro-inflammatory cytokine expression by suppression of p38 MAPK phosphorylation in heat-killed *P. acnes*-treated HaCaT cell. This in vitro action of anti-inflammatory property of bee venom study can be further translated into in vivo study of human subjects with acne vulgaris.

In the current study, we analyzed acne lesions using Image Pro software which is an objective technique to determine the condition of face in terms of irritation and appearance of acnes. This image analysis correlated well with the clinical findings. Moreover, a significant difference in visual scale was observed after 3 weeks with PBV™ serum, indicating the faster effect of PBV™ serum on acne improvement.

Our findings suggest that bee venom containing serum provided a greater efficacy in terms of acne lesion counts. PBV™ may be a good candidate

compound for developing therapeutic drugs for the treatment of acne vulgaris. Long-term treatment of acne with PBV™ serum could be safe, because dermal irritation potential of PBV™ is negligible.[11]

Acknowledgements

This work supported by a grant (Code #: PJ01132503 from the Next-Generation BioGreen 21 Program) Rural Development Administration, Republic of Korea. Manuka Doctor ApiClear Foaming Cleanser and Manuka Doctor ApiClear Skin Treatment Serum were kindly supplied by Manuka Doctor for this research trial.

Declaration of interest

There is no conflict of interest to declare.

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