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Safety of essential bee venom pharmacopuncture as assessed in a randomized controlled double-blind trial

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ABSTRACT

Ethnopharmacological relevance: While bee venom (BV) pharmacopuncture use is common in Asia, frequent occurrence of allergic reactions during the treatment process is burdensome for both practitioner and patient.
Aim of the study: This study compared efficacy and safety in isolated and purified essential BV (eBV) pharmacopuncture filtered for phospholipase A2 (PLA2) and histamine sections, and original BV to the aim of promoting safe BV pharmacopuncture use.
Materials and methods: In in vitro, we examined the effect of BV and eBV on nitric oxide (NO) production induced by lipopolysaccharide (LPS) in RAW 264.7 macrophages, and clinically, 20 healthy adults aged 20–40 years were randomly allocated and administered eBV 0.2 mL and BV pharmacopuncture 0.2 mL on left and right forearm, respectively, and physician, participant, and outcome assessor were blinded to treatment allocation. Local pain, swelling, itching, redness, wheals, and adverse reactions were recorded by timepoint.
Results: eBV and BV exhibited similar inhibitory effects on NO production. Also, in comparison between eBV and BV pharmacopuncture administration areas on each forearm, eBV displayed significantly lower local pain at 24 h post-administration (P=0.0062), and less swelling at 30 min (P=0.0198), 2 (P=0.0028), 24 (P=0.0068), and 48 h post-administration (P=0.0253). eBV also showed significantly less itching at 24 (P=0.0119), 48 (P=0.0082), and 96 h (P=0.0141), while redness was significantly less at 30 min (P=0.0090), 6 (P=0.0005), and 24 h ($P < 0.0001$). Time-by-treatment interactions were statistically significant for itching and redness ($P < 0.001$, and $P < 0.001$, respectively), and all original BV pharmacopuncture administered regions showed a tendency toward more severe itching and redness in later measurements.
Conclusions: eBV and BV displayed comparable anti-inflammatory effects, and eBV pharmacopuncture presented less local allergic reactions.

1. Introduction

Bee venom (BV) is one of the most commonly utilized animal venoms and comprises various chemical agents that induce allergic responses (Czarnetzki et al., 1990). BV therapy, where BV is used as a medicinal intervention, is available worldwide, but is primarily used in Asia, Eastern Europe, and South America (Alqutub et al., 2011). In particular, BV pharmacopuncture is a type of applied acupuncture treatment that uses BV extracted from the venom sac of live honeybees (western species of worker bee, Apis mellifera), which is injected at appropriate dose at acupuncture points selected through syndrome differentiation as a pharmacopuncture preparation to the aim of simultaneous acupuncture and pharmaceutical BV stimulation (Korean Pharmacopuncture Institute, 1999).

BV has pain-relieving anti-inflammatory effects (Koh, 1992; Kwon and Koh, 1998), affects the immune system (Kwon et al., 1997), and has been shown to possess significant therapeutic effects on degenerative knee (Lee et al., 2003c; Wang et al., 2002) and hip joint osteoarthritis (Kim et al., 2001), rheumatoid arthritis (Lee et al., 2003a, 2003b), and lumbar intervertebral disc herniation (Jun et al., 2003; Kim et al., 2005; Lee et al., 2004). BV pharmacopuncture is also recommended in the major clinical practice guidelines published by the Korea Institute of Oriental Medicine for lumbar intervertebral disc herniation treatment by Korean Medicine doctors (KMDs) (Evidence-based Korean Medicine Clinical Practice Guideline Development Committee for Lumbar Herniated Intervertebral Disc Korea Institute

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of Oriental Medicine and the Society of Korean Medicine Rehabilitation, 2014).

In addition, pathways involved in the various oxidative stress, allergy, and inflammation-related effects of BV are being studied. In mechanism studies on oxidative stress, BV inhibited lipid peroxidation (LPO) levels, which were used as a marker to indicate membrane damage (Rekka et al., 1990), and superoxide production (Somerfield et al., 1984). Regarding phosphatase activity, BV was shown to induce alkaline phosphatase (ALP) and acid phosphatase (ACP) expression in serum levels (Hoffman, 2006; Ishay et al., 1977). Also, BV has been reported to exert anti-inflammatory properties by blocking expression of inflammatory mediators such as NO, COX-2, TNF-α, and IL-1 in inflammation-related mechanism studies (Son et al., 2007).

BV contains 40 main components, and of these constituents, peptides such as melittin, apamin, adolapin, and mast cell degranulating peptide (MCD peptide) play major roles in treatment. Melittin (a 26 amino acid peptide with the following sequence: Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gin-NH2 which is the principal component of BV) takes up approximately 50% of dried BV, and is known to possess excellent pain-relieving and anti-inflammatory effects (Banks and Shipolini, 1986; Schmidt, 1986). However, phospholipase A2 (PLA2) and histamine are known to incur allergic reactions. Various allergic responses occurring during treatment impose a considerable burden on both practitioner and patient, and cases where the patient sustains anaphylactic shock from BV treatment as a systemic immediate hypersensitive response is the most severe type of hypersensitivity to BV (Heo, 1993; Hwang and Lee, 2000; Youn, 2005). To this aim, essential BV (eBV) was developed by removing macromolecular enzymes including PLA2 and micromolecular substances including histamine to maximize active substance content and effectively block possible sources of allergic response for safe BV pharmacopuncture practice.

The basic research part of this study therefore investigated whether the original BV type and newly developed eBV differed in anti-inflammatory response at the same dose in vitro, and the randomized controlled trial (RCT) part compared eBV safety through local allergic response to promote wider clinical application of BV pharmacopuncture and Korean medicine.

2. Materials and methods

2.1. In vitro study

2.1.1. Chemicals

Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), sodium pyruvate, $_{L}$-glutamine, antibiotics-antimycotics solution, and trypsin-EDTA were purchased from Invitrogen Co. (Grand Island, NY, USA). Lipopolysaccharide (LPS), 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), bicinchoninic acid (BCA), *N*-(1-naphthyl) ethylenediamine dihydrochloride, sulfanilamide, phosphoric acid, dimethyl sulfoxide (DMSO), melittin, histamine, PLA2, and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise indicated.

Dried BV was commercially purchased (Chung Jin Biotech Co. Ltd., Ansan, Korea). For quantitative analyses, water, acetonitrile (ACN), and methanol (MeOH) of high performance liquid chromatography (HPLC) grades were purchased from JT Baker (Phillipsburg, NJ, USA). Trifluoroacetic acid (TFA) was purchased from Junsei Chemical (Tokyo, Japan).

2.1.2. Cell culture

Mouse macrophage RAW 264.7 cells obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) were cultured in DMEM supplemented with 10% heat-inactivated FBS, 100 unit/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B. Cells was incubated at 37 °C with 5% CO_2 in a humidified atmosphere.

2.1.3. Nitrite assay

To evaluate the inhibitory effect of BV and eBV on LPS-induced NO production, RAW 264.7 cells were plated in a 24-well culture plate (5×10^5 cells/mL), and incubated for 24 h. Cells were washed with PBS, fresh medium was added, and the cells were then incubated with 1 μg/mL LPS in the presence or absence of BV or eBV. After 20 h of incubation, media was collected and analyzed for nitrite accumulation as an indicator of NO production by Griess reaction. Briefly put, 180 μL of Griess reagents (0.1% *N*-(1-naphthyl) ethylenediamine dihydrochloride in H_2O and 1% sulfanilamide in 5% H_3PO_4) was added to 100 μL of each supernatant from LPS or BV or eBV-treated cells in 96-well plates. The absorbance was measured at 540 nm, and nitrate concentration was determined by comparison with a sodium nitrite standard curve. Percent inhibition was expressed as [1-(NO level of BV or eBV/NO levels of vehicle treated control)]×100. The IC_{50} value, the sample concentration resulting in 50% inhibition of production, for BV, eBV, and melittin which was used as a positive control was determined using non-linear regression analysis (% inhibition versus concentration).

2.1.4. Cytotoxicity assay

To assess the cytotoxic effect of BV, eBV, and melittin in RAW 264.7 cells in the assay condition, MTT assay was performed. After the Griess reaction, MTT solution (final concentration of 500 μg/mL) was added to each well and further incubated for 4 h at 37 °C. The media was discarded, and DMSO was added to each well to dissolve generated formazan. Absorbance was measured at 570 nm, and % survival was determined by comparison with the control (LPS only treated cells).

2.2. Clinical trial

2.2.1. Design and participants

This study was a participant, physician, and outcome assessor-blinded RCT. Subjects were healthy individuals aged 20–40 years and participant inclusion and exclusion criteria were as follows:

2.2.1.1. Inclusion criteria.

1) Individuals with no experience of previous BV pharmacopuncture administration.
2) Healthy male or female adults of age 20–39 years.
3) Individuals who give voluntary written consent to participate in the trial with no significant issues with expression of opinion.

2.2.1.2. Exclusion criteria.

1) Individuals taking conventional medicine for chronic disorders such as hypertension or diabetes.
2) Individuals with history of allergic dermatitis.
3) Individuals with sensory defect.
4) Individuals with psychopathic disorders.
5) Individuals with history of hypersensitivity to bee stings.
6) Individuals with fever symptoms (e.g. from colds).
7) Pregnant individuals.
8) Individuals who have apprehension regarding the trial or do not wish to participate.

Twenty participants were recruited through advertisements at Jaseng Hospital of Korean Medicine in Seoul, Korea, and the recruitment period was from January 21st to January 31st, 2015. Eligible individuals who gave voluntary and informed written consent to