Journal of Pharmacopuncture

ISSN 2093-6966 [Print], ISSN 2234-6856 [Online]
Journal of Pharmacopuncture 2016;19[3]:225-230
DOI: http://dx.doi.org/10.3831/KPI.2016.19.023

Original article

Bee Venom (*Apis Mellifera*) an Effective Potential Alternative to Gentamicin for Specific Bacteria Strains

- Bee Venom an Effective Potential for Bacteria-

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Key Words

antibacterial activity, *Apis mellifera*, bee venom, disc-diffusion

Abstract

Objectives: Mellitine, a major component of bee venom (BV, *Apis mellifera*), is more active against gram positive than gram negative bacteria. Moreover, BV has been reported to have multiple effects, including antibacterial, antivirus, and anti-inflammation effects, in various types of cells. In addition, wasp venom has been reported to have antibacterial properties. The aim of this study was to evaluate the antibacterial activity of BV against selected gram positive and gram negative bacterial strains of medical importance.

Methods: This investigation was set up to evaluate the antibacterial activity of BV against six grams positive and gram negative bacteria, including *Staphylococcus aureus* (*S. aureus*), *Salmonella typhimurium*, *Escherichia coli* (*E. coli*) O157:H7, *Pseudomonas aeruginosa*, *Burkholderia mallei* and *Burkholderia pseudomallei*. Three concentrations of crude BV and standard antibiotic (gentamicin) disks as positive controls were tested by using the disc diffusion method.

Results: BV was found to have a significant antibacterial effect against *E. coli*, *S. aureus*, and *Salmonella typhyimurium* in all three concentrations tested. However, BV had no noticeable effect on other tested bacteria for any of the three doses tested.

Conclusion: The results of the current study indicate that BV inhibits the growth and survival of bacterial strains and that BV can be used as a complementary antimicrobial agent against pathogenic bacteria. BV lacked the effective proteins necessary for it to exhibit antibacterial activity for some specific strains while being very effective against other specific strains. Thus, one may conclude, that *Apis mellifera* venom may have a specific mechanism that allows it to have an antibacterial effect on certain susceptible bacteria, but that mechanism is not well understood.

Received: Mar 23, 2016 **Reviewed:** Apr 05, 2016 **Accepted:** Jul 31, 2016

1. Introduction

Bee venom (BV, *Apis mellifera*) comprises a very complex mixture of active peptides, enzymes and amines [1]. The antimicrobial activity of BV has been documented for both gram negative and gram positive bacteria, including *Escherichia coli* (*E. coli*) and *Salmonella* spp, *Enterobacter cloacae*, *E. coli* and *Citrobacter freundii*, *Staphylococcus aureus* (*S. aureus*), and coagulase-negative *Staphylococcus* and *E.coli*. The majority of bacteria, such as *Streptococcus*, *Pseudomonas*, *Salmonella*, *Escherichia*, *Burkholderia*, have developed several ways to resist antibiotics. Such bacteria are becoming a serious clinical problem throughout the world. Thus, new effective antibacterial agents with new antibacterial mechanisms need to be continuous-

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ly developed [2].

Natural products such as venoms are an important source of pharmaceutical compounds [3, 4]. BV from honey bees has long been used as a complementary medicine to treat an array of conditions [5]. BV contains a great variety of biologically-active proteins responsible for various pathological effects. Venoms include toxins, which are high-potency compounds with selective and specific activities. They can be useful and valuable as pharmacological tools in drug research, as potential drug design templates, and as therapeutic agents [6, 7]. BV's major component, mellitine, is more active against gram positive than gram negative bacteria. Also, the venom of wasps has been reported to have antibacterial properties [8, 9].

Well documented is the fact that the extensive use of antibiotics over the past six decades in our hospitals and communities has led to an increased prevalence of bacteria with acquired resistance to the antibiotics typically used to treat infections, giving rise to a critical need for the development of new approaches to the treatment of bacterial infections [10].

In the present study, we investigated the antimicrobial activity of BV and of a standard antibiotic gentamicin against selected bacteria strains.

2. Material and Methods

Lyophilized BV was prepared at the Venomous Animals Department, Razi Vaccine and Serum Institute, and was kept at – 20°C.

The gram positive bacterium *S. aureus* (ATCC 25923) and gram negative bacteria, including *Pseudomonas aeruginosa* (ATCC 27853), *E. coli* O157:H7 (ATCC 25923), *Salmonella typhimurium* (ATCC 25923), *Burkholderia mallei*, (RTCC:2375), and *Burkholderia pseudomallei* (RTCC:2375), were prepared at the Department of Tuberculin and Mallein Production and Research, Razi Vaccine and Serum Research Institute, Karaj, Iran.

The standard antibiotic gentamicin was purchased from Liofilchem S.r.1 Diagnostic Company (Italy) and was used for comparison with the BV.

The antibacterial effects were tested by using disc diffusion assays [11, 12]. Pure cultures were prepared by sub-culturing the test strain in 10 mL of brain heart infusion broth (BHI broth) following incubation at 37°C for 24 hours. The concentration of the resulting culture was determined by preparing serial dilutions and surface plating on standard plate count (SPC) agar.

The culture media was diluted and adjusted to 0.5 Mc-

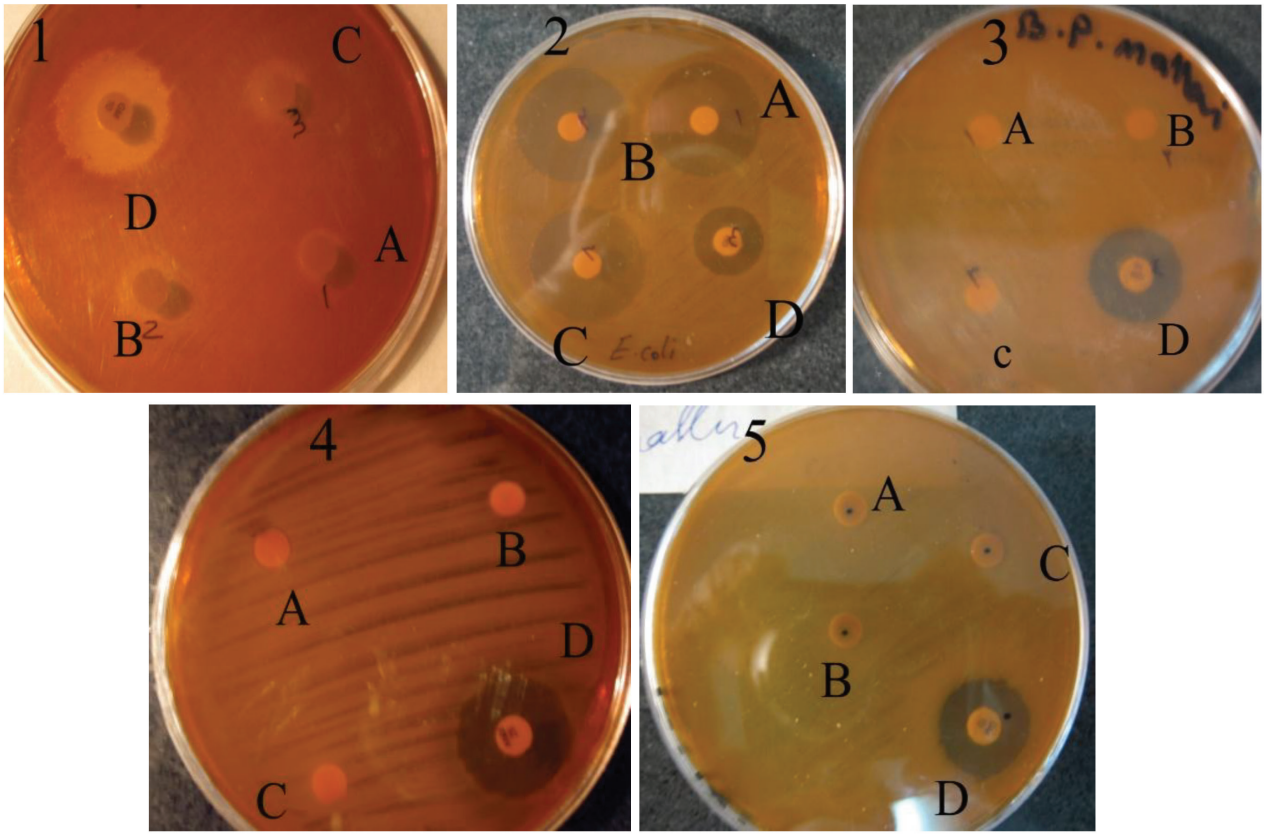

Figure 1 Antibacterial effect of crude bee venom in three concentrations, A (25 μg), B (35 μg) and C (45 μg), compared to that of the standard antibiotic gentamicin (D) (n = 6) against (1) *Salmonella typhimurium*, (2) *Escherichia coli*, (3) *Burkholderia pseudomallei*, (4) *Pseudomonas aeruginosa*, and (5) *Burkholderia mallei*.

Table 1 *In vitro* antibacterial activity of *Apis Mellifera* crude venom tested by using disc-diffusion and compared to the standard antibiotic gentamicin. Each result is presented as mean ± inhibition zone in mm (n = 6).

Microbial strain Concentration of venom/ gentamicin		*Salmonella typhimurium*	*Escherichia. coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Burkholderia mallei*	*Burkholderia pseudomallei*
Concentration venom (μg)	25 μg	10.51 ± 0.62	26.66 ± 1.505	11.46 ± 0.87	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	35 μg	15.07 ± 0.603	31.305 ± 1.02	13.24 ± 0.98	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	45 μg	15.88 ± 0.73	32.46 ± 0.67	15.51 ± 1.077	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Gentamicin (10 μg/mL)		19 ± 0.0	20 ± 0.0	18 ± 0.0	18 ± 0.0	20 ± 0.0	18 ± 0.0

Farland standards containing 1.5×10^8 CFU/mL so that the same dose of could be inoculated in repeated experiments. The absorbance of the culture medium at 600 nm was determined by using a spectrophotometer. A sterile cotton swab was used for spreading diluted culture samples on mueller-hinton (MH) agar plates. Sterile blank paper discs (7-mm diameter) were then placed on the MH agar's surface, and a venom sample was added to each disc; five replicates were made. Antibiogram disks including gentamicin (10 μg/disk) were added as positive controls. The plates were incubated at 37°C for 24 to 28 hours, and the zones of inhibition were measured. The experiments were performed at least five times. The means and standard deviations of the data collected for each experiment were calculated using Microsoft Excel.

For the preparation of the BV, two hundred live bees were collected from a bee hive. The stingers with the venom sacs were removed by force and placed in a jar, after which they were lyophilized. The procedures were performed at the Venomous Animals Department, Razi Vaccine and Serum Institute, and the final products were stored – 20°C. The bacterial and test control isolates used in the present study were collected from the National Research Laboratory of Razi Vaccine and Serum Research Institute, Karaj, Iran.

These isolates were identified based on standard microbiological techniques, and drug susceptibility tests were done following the standard agar disc diffusion method.

3. Results

To evaluate the antibacterial activity of BV, we used the disk diffusion method with a 20-μL concentration of proteins. The prepared BV in a dose of 25, 35, or 45 μg was added to a disk, as was the standard antibiotic gentamicin in a dose of 10 μg/mL, and six independent experiments were performed. BV demonstrated antibacterial activity against *E. coli*, *S. aureus*, and *Salmonella typhimurium* at all three concentrations (Fig. 1). Moreover, with increasing BV concentration, the inhibition zone increased (Fig. 2).

The venom concentration of 45 μg showed the highest inhibition zone against *E. coli* (32.46 ± 0.67), *S. aureus* (15.51 ± 1.077) and *Salmonella typhimurium* (15/88 ± 0/73) (Table 1), and BV had a more significant antibacterial activity against *E. coli* in the medium than it did against either of the two other strains of bacteria.

The present examination also showed that the antibacterial activity of BV at the three concentrations against *E. coli* was more significant than it was for the standard antibiotic gentamicin at 10 μg/mL (Fig. 3). However, the antibacterial activities of BV against *S. aureus*, and *Salmonella typhimurium* at the three concentrations were less than the effect of the standard antibiotic gentamicin at 10 μg/mL (Figs. 4, 5). Furthermore, BV was found to have no observable effect on the other tested bacteria whereas the standard antibiotic gentamicin was effective against those bacteria (Fig. 6 and Table 1).

4. Discussion

The biochemical, antimicrobial and pharmacological effects of BV have been reported in several studies [13-15]. The antimicrobial activity of honey BV may be due to the

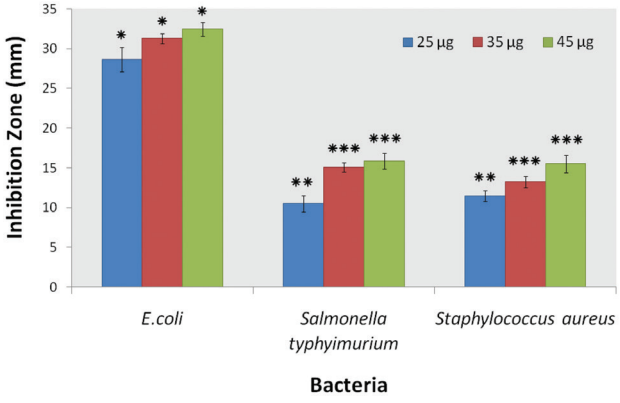

Figure 2 Inhibition zone at the three concentration of bee venom against *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus* (sig 0 < 0.05). This fig shows that bee venom has a more significant antibacterial activity against *Escherichia coli* in a medium than the two other strains of bacteria do (n = 6).

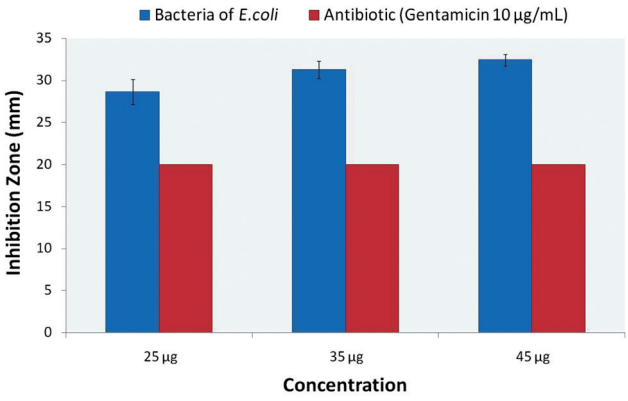

Figure 3 Inhibition zone of bee venom in three concentrations against *Escherichia coli* in a medium in comparison to that of the standard antibiotic gentamicin (n = 6).

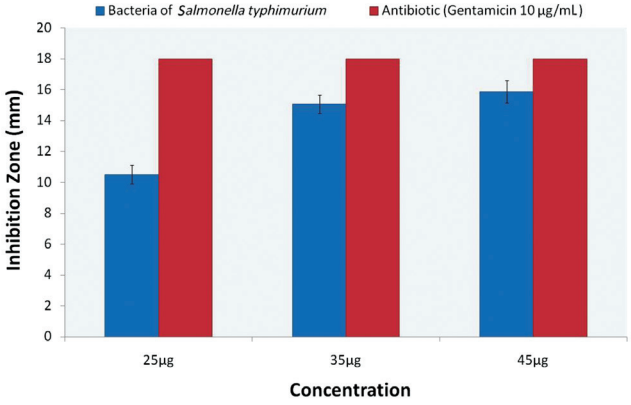

Figure 4 Inhibition zone of bee venom in three concentrations against *Salmonella typhimurium* in a medium in comparison to that of the standard antibiotic gentamicin (n = 6).

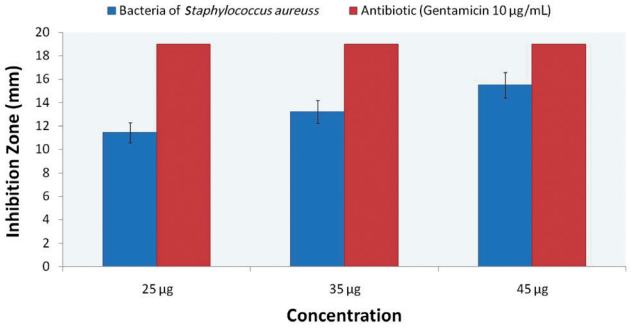

Figure 5 Inhibition zone of bee venom in three concentrations against *Staphylococcus aureus* in a medium in comparison to that of the standard antibiotic gentamicin (n = 6).

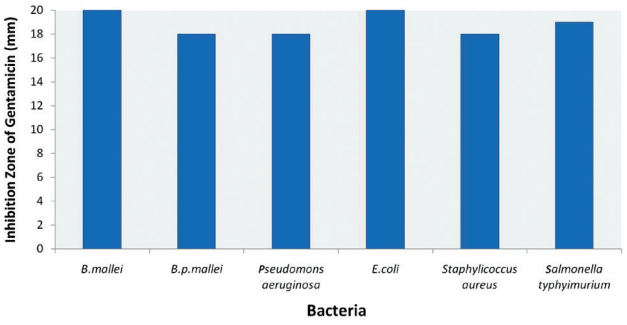

Figure 6 Inhibition zone shown by Gentamicin against the selected bacteria.

presence of several peptides, such as melittin, apamin, adolapin, mast-cell-degranulating peptides, enzymes, biologically-active amines and non-peptide components [16]. Cujova *et al* [17] reported that honey BV contained melittin, which is more active against gram positive than gram negative bacteria.

The relative sensitivities of the bacteria were qualitatively estimated by measuring the zones of inhibition. When Ortel and Markwardt quantitatively determined the zones of inhibition, they found that gram positive organisms were sensitive to lower concentrations of BV than gram negative organisms were [18].

Accordingly, BV clearly does not have an antibacterial effect on all the bacteria we tested, although against specific bacteria tested, it does have a more significant antibacterial activity than the standard antibiotic gentamicin does. The antibacterial effect of BV may be due to the presence of peptides, enzymes, biologically-active amines, and non-peptide component [19], and these compounds may interact with specific molecules of some bacteria while not affecting others. Phospholipase A_2 (PLA_2) has been reported to have an antibacterial effect [20, 21]. Although BV contains PLA_2, which may be responsible for its antibacterial properties, it also contains melittin, which may contribute to that antibacterial effect.

The data obtained in this study confirm the results of a previous work that BV can inhibit bacterial growth. More gram negative organisms than gram positive organisms are sensitive to BV, and other drug-resistant microorganisms may exhibit a similar property.

Investigators have reported that the levels of antibacterial activity against gram negative and gram positive bacteria may be different, depending on the antibacterial agents [22].

The results in our study are in general agreement with those found by Kondoand Kanai [23], who reported that mycobacteria and staphylococci, but not *E. coli*, were affected by the BV fraction (melittin). Also, Hegazi *et al* [24] showed that bee products were less effective against *E. coli*, and Rybak [25] reported that BV + kanamycin exhibited synergistic activity against a kanamycin-resistant strain of *S. aureus*.

If the influence of BV on other pathogens is to be determined, further studies using a wider range of gram posi-

tive and gram negative bacteria and other concentrations of BV are needed. The experimental data from the current study confirmed the results of all previous works, which suggested that BV could inhibit the growth of some bacterial strains, but not others.

5. Conclusion

BV has been established to have antibacterial effects against gram-positive bacteria. Furthermore, in this study, BV was found to inhibit the growth and survival of specific bacterial strains, but not others; thus, one can conclude that BV may be an effective complementary antimicrobial agent for use against specific pathogenic bacteria. Moreover, the research on BV may well lead to the future design of novel pharmaceuticals.

Acknowledgment

The authors would like to thank all the members of the Department of Venomous Animals and Antivenom Production. The authors would also like to thank all their colleagues in the Department of Tuberculin and Mallein Production and Research for their great help.

Conflict of interest

The authors declare that there are no conflict of interest.

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