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Antimicrobial activities of honey bee venom against pathogens isolated from clinical bovine mastitis in Korea

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Introduction: Bovine mastitis is one of the most problematic bacterial diseases in the dairy industry. Antibiotic therapy continues to play an important role for treatment. However, appearance of resistant bacteria causes therapy failure and triggers the exploration of alternative therapy for the bovine mastitis. In Korea, honey bee (*Apis mellifera*, L) venom therapy (*apitherapy*) has been elucidated therapeutic value for farm animals with bacterial diseases and reported to be as effective as antibacterial drugs. However, there is little report about honey bee venom application for alternative treatment of clinical bovine mastitis in Korea. This study was carried out to evaluate the antibacterial activity of honey bee venom against the bacterial mastitis pathogens isolated from Korean dairy farms.

Materials and Methods: This study was performed by using three honey bee venom products and 44 strains of bacteria isolated from infected cows' mammary quarters with mastitis in Korea; *Staphylococcus aureus* (10 strains), Coagulase-negative *Staphylococcus* (CNS, 7 strains), E. coli (7 strains), *Serratia marcescens* (5 strains), *Klebsiella pneumoniae* (5 strains), *Pseudomonas aeruginosa* (5 strains) and *Citrobacter freundii* (5 strains). The concentration of major active components of bee venom, melittin, apamin and phospholipase A2, were analyzed by HPLC and antimicrobial activity and potent were confirmed and compared among the three bee venom products by cylinder agar plate method and determination of minimum inhibitory concentration (MIC). Time kill assays and electron scanning microscopy were used for observation of pore forming on cell membrane during the bacterial inhibition of the sensitive strains over time.

Results: The concentrations of three major active components of two venom products were two times more than that of another one. All three products effectively inhibited the growth of *S. aureus*, and CNS and partially inhibited that of *E. coli* (2 of 7 strains) while didn't inhibit those of the others (at the over 500 μg/ml of concentrations). MIC of *S. aureus*, CNS and *E. coli* were 62.5~125, 62.5~250 and 62.5~250 μg/ml respectively. However the pore forming on cell membrane wasn't observed by electron scanning microscopy at the time kill assay for the sensitive strains.

Conclusions: In this study, the concentration of three major active components had a little frustration among three venom products, but the antimicrobial activities were not different statistically. It was clearly demonstrated that the honey bee venom inhibited the growth of seventeen gram positive bacteria strains and two gram negative strains isolated from bovine mastitis in Korea. However, the pore forming on cell membrane wasn't observed by using bee venoms and sensitive gram positive bacteria. It is needed to undergo more experimental investigation to ascertain the mechanism of action of antimicrobial activity of bee venom.

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