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The effect of gamma-irradiation and heating on the antibacterial activity of honey bee (Apis mellifera) venom



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The use of honeybee (Apis mellifera L.) venom as a topical antibacterial agent has been rediscovered by the medical profession in recent times. As a traditional alternative medicine approach, honeybee venom (HBV) has been utilized to relieve pain and to treat inflammatory diseases since ancient ages (Billingham et al., 1973) thus apitherapy came into use. Honeybee venom, as a therapeutic modality in use, has been extensively researched and practiced in Korea, focusing on clinical applications as a meridian therapy (Yin et al., 2005). HBV has been reported to have an antibacterial effect, without interfering with other drugs and it is not expensive (Boutrin et al., 2008). The polypeptide melittin is the main component of honeybee venom (Piek, 1986). It accounts for more than 40% by weight of dry venom. Melittin has a powerful antibacterial and antifungal activity against many kinds of organisms (Piek, 1986), and it is worth considering whether HBV can be sterilized for use a

medical supplies. To date, there have been no reports of the effects of sterilizing with either gamma-irradiation or autoclaving on the antibacterial activity of HBV. Therefore this study was undertaken to determine the effects of gamma-irradiation and autoclaving on the antibacterial activity of HBV.

HBV was collected by a bee venom collector (ChungJin, Korea) from experimental colonies of honeybees (A. mellifera) maintained at the National Academy of Agricultural Science, South Korea. The collected HBV was diluted in cold water and then centrifuged at 10,000 g for 5 min at 4°C to discard residues from the supernatant. HBV was lyophilized by a freeze dryer. The sample of HBV was dispensed into screw-capped 25 mm X 50 mm glass sample vials (100-200 mg in each) for processing through a commercial sterilizing plant which guaranteed to subject HBV to 25 kGy of gamma-irradiation for 3 hr. The sample of HBV was prepared in the

Table 1. Comparison of the antibacterial activity of honeybee venom (HBV) before and after sterilization.

	Minimum inhibitory concentration (ug/mL)	
Sample	Escherichia coli	Staphylococcus aureus
Untreated HBV	0.25 ± 0.03	0.06 ± 0.02
HBV after 25 kGy irradiation x 3 h	0.25 ± 0.07	0.06 ± 0.05
HBV after autoclaving at 121°C x 15 mins	0.25 ± 0.08	0.06 ± 0.05

The values are expressed as the mean \pm s.e. (n=3) from three independent experiments.

same way, and autoclaved for 15 min at 121°C. The antibacterial activity of the HBV samples was assayed to determine the minimum inhibitory concentration (MIC). Using *Escherichia coli* (ATCC 14169) and *Staphylococcus aureus* (ATCC 6538) were obtained from the Korean culture centre of microorganisms, South Korea. MIC for HBV was determined by broth dilution method (Wu and Hancock, 1999). The inhibition of bacterial growth was determined by absorbance using a microplate reader at 405 nm, and MIC was recorded as the lowest concentration of HBV that inhibited the growth.

The antibacterial activity of the HBV before and after sterilization is shown in Table 1. These results show that there was no significant loss of antibacterial activity when HBV was either gamma-irradiated or autoclaved.

The effect of sterilization on the antibacterial activity of HBV used has not been discussed in any published reports on the use of HBV for medical purposes. This study has shown that exerting a 25 kGy dose for 3 hours or autoclaving at 121°C for 15 mins (as used in commercial sterilization of medical material) does not destroy the antibacterial activity of the HBV. More research into the effect of sterilization on composition of HBV is needed.

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