Original Article

JVC Journal of Veterinary Clinics

pISSN 1598-298X • eISSN 2384-0749
J Vet Clin 2024;41:370-376
https://doi.org/10.17555/jvc.2024.41.6.370

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Therapeutic Effect of Bee Venom in Holstein Dairy Cows with Clinical Mastitis

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Abstract This study examines the therapeutic effects of bee venom on dairy cows with clinical mastitis, a common and economically significant disease in the dairy industry. While mastitis is traditionally managed with antibiotics, the increasing problem of antibiotic resistance calls for the exploration of alternative treatments. Known for its anti-inflammatory, antimicrobial, and immunomodulatory properties, bee venom was evaluated as a potential alternative. Fifty-one Holstein Friesian cows from five dairy farms, all diagnosed with clinical mastitis, participated in the study. Administered intramammarily for seven consecutive days, the diluted bee venom from New Techniques Laboratory Ltd. was monitored for its effects on clinical symptoms, somatic cell counts, and bacterial cultures at baseline and on days 3, 5, 7, 10, and 14 post-treatment. The results indicate that bee venom is particularly effective against gram-positive bacteria, with notable recovery observed in *Staphylococcus saprophyticus* infections within 5 days. However, its efficacy against gram-negative bacteria, especially *E. coli*, was less pronounced, showing improvement in 50% of *E. coli* cases by day 14. No therapeutic response was noted in cases involving *Klebsiella pneumoniae*. These findings support the potential of bee venom as an alternative treatment for mastitis, particularly against gram-positive infections but highlight the necessity for further research to improve its effectiveness against gram-negative bacteria and to explore its role in managing antibiotic-resistant strains.

Key words clinical mastitis, bee venom, dairy cow, therapeutic effect, bacteria.

Received November 29, 2024 / Revised December 6, 2024 / Accepted December 6, 2024

Introduction

Controlling disease outbreaks in livestock during the production process poses a significant challenge for the agricultural sector (43). Diseases not only inflict suffering on animals but also lead to substantial economic losses for farmers due to decreased income and increased expenses (29). A notable example of such a disease is mastitis in dairy cows, which is prevalent on dairy farms (1,3). Mastitis induces pain and fever in cows, and farmers incur losses from decreased milk production, quality, disposal costs, culling, treatment expenses for infected cattle, and additional labor costs (18,21,26). To mitigate the impact of mastitis, both veterinarians and farmers engage actively in its prevention and treatment. Mastitis is categorized into clinical and subclinical forms based on the severity of the inflammation. Clinical mastitis is distinguished by visible symptoms such as udder swelling, redness, and milk that appears flaky, clotted, or watery (2,4,24,30,51). The primary pathogens responsible for clinical mastitis include *E. coli*, Klebsiella (Gram-negative), *Staphylococcus aureus*, and *Streptococcus* species (Gram-positive) (6,16,19).

To treat mastitis in dairy cows, a drug susceptibility test is performed, followed by the administration of an effective drug to which the pathogen is sensitive (28,46,47). Common antibiotics for treating mastitis include penicillin, ampicillin, cloxacillin, gentamycin, kanamycin, and bacitracin, typically administered as ointments injected into the udder (41,46,53). However, the extensive use of antibiotics has led to increased antibiotic resistance, complicating the selection of effective treatments and necessitating stronger antibiotics for resistant strains (8,38,42). This overreliance on antibiotics poses risks to consumer health and contributes to antibiotic resistance, leading several countries to impose restrictions on antibiotic use (5,15,34,40,42,44). In response, Korea has explored the use of bee venom as an alternative approach to reduce antibiotic dependency in livestock (22).

Bee venom, traditionally used in Oriental medicine, is known for its anti-inflammatory, pain-relieving, anticancer, and multiple sclerosis treatment effects. It also exhibits antimicrobial and immunomodulatory properties (7,23,25,54). Given these therapeutic properties, bee venom is being explored as a treatment for mastitis in dairy cows, offering potential benefits such as reduced antibiotic usage, shorter lactation periods, enhanced productivity, and improved animal welfare (12,52). Two primary methods of bee venom application in mastitis treatment involve direct injection into acupuncture points on the cow's body using live bees, or via needles delivering purified bee venom (20,35). However, these methods can cause allergic reactions, pain, discomfort, and stress related to acupuncture and injections (10,36). To mitigate these issues, a more targeted approach has been proposed, involving direct injection of bee venom into the udder via a nipple needle for treating mastitis (22). Despite the promising properties of bee venom, few studies have evaluated its therapeutic effects on dairy cows with clinical mastitis (23). Thus, the purpose of this study is to investigate the therapeutic effects of bee venom in dairy cows suffering from clinical mastitis.

Materials and Methods

Animals

The study examined dairy cows diagnosed with clinical mastitis across five dairy farms, each milking between 50 and 100 cows, located in the Imsil and Sunchang regions of Jeollabuk-do. A total of 55 quarters from 51 Holstein Friesian cows with naturally occurring clinical mastitis were included in this analysis. Clinical mastitis was identified using a positive result from the California Mastitis Test (CMT), coupled with the presence of abnormal milk characteristics (e.g., changes in color, viscosity, or consistency) and abnormal udder features (such as swelling, heat, pain, or redness). The farms involved in this study milked their cows twice daily, at 5 am and 5 pm, and ensured regular monthly reproductive care by a veterinarian.

Treatment of bee venom

Bee venom used in this study was sourced from New Techniques Laboratory Ltd. (Georgia, PK). The composition of the bee venom components is depicted in Table 1. The venom was freeze-dried and stored in a refrigerator. Before use, 50 mg of bee venom was diluted in 50 mL of physiological saline. The diluted venom was used immediately, and any remaining solution was discarded. A total of 12 mL of this solution was administered intramammarily through the teat canal once daily for 7 days.

Table 1. Composition of bee venom

Substances	Description (%)
Loss mass when dried	6.0
Water insoluble substances	3.0
Total ash	1.2
Activity of phospholipase A_2 (IU/mg)	18.5
Activity of hyaluronidase (IU/mg)	13.0
Apamin	13.2
Phospholipase A_2	24.6
Melittin	58.6

Observation of clinical symptoms

Clinical symptoms were meticulously observed and documented immediately before the treatment commenced. Post-treatment observations included daily monitoring of abnormal milk, udder health, and dietary habits. These observations were systematically recorded at milking times until the final sample was collected.

Sample collection

Milk samples were collected before the treatment and on days 3, 5, 7, 10, and 14 after the administration of bee venom. Collection was performed away from the milking area. Initially, the teats were disinfected with alcohol-soaked towels and then dried with a clean towel. The first few streams of milk were discarded to cleanse the teat canal, after which milk was collected into two 50 mL tubes. All samples were stored at 4°C and immediately transported to the College of Veterinary Medicine, Chungbuk National University.

Somatic cell grade

Somatic cell count (SCC) was categorized into 4 grades based on the CMT results, with slight modifications to the method described by Sonnewald-Daum et al. (48). Specifically, a CMT result exceeding 5 million (clumps, highly viscous, and no longer possible to discard) was classified as grade 4; results ranging from 800,000 to 5 million (gel) were classified as grade 3; results between 200,000 and 800,000 (traces) were classified as grade 2; and results under 200,000 (negative) were classified as grade 1. Quarters with a CMT response of ≥Grade 2 and clinical signs of mastitis were considered to indicate clinical mastitis. Milk from quarters with a CMT response of ≥1+ was aseptically collected, immediately cooled on ice, and transported to the laboratory for SCC measurement. Somatic cells were measured using a somatic cell counter (Foss 300®, Foss Electric Ltd, Denmark).

Bacteriological examination

Mastitis pathogens were isolated by inoculating 23 μL of milk onto a 5% sheep blood agar plate, which was then incubated at 37°C for 48 hours. After incubation, bacterial colony characteristics, hemolysis patterns, and Gram staining were observed for preliminary identification of the bacteria. Further identification was conducted by the National Veterinary Research and Quarantine Service.

Following incubation in 20 mL of Trypticase Soy Agar at 37°C for 18 hours, single colonies were picked and transferred to 7 mL of Trypticase Soy Broth. This broth was then incubated in a shaking incubator at 37°C for 16 to 18 hours. After incubation, the bacterial solution was adjusted to a 0.5 McFarland standard using sterilized saline and a turbidity meter. The solution was then injected under vacuum into a GPI card for VITEK 1 analysis. After 8 hours, the bacterial identification was determined based on the results.

Definition of clinical and bacteriological cure

Therapeutic effectiveness was evaluated based on clinical and bacteriological cures (31). Clinical cure was defined as the absence of abnormal milk (e.g., clots or flakes) and a normal udder appearance (i.e., no redness or swelling) 14 days after treatment. Bacteriological cure was defined as the absence of growth of the previously isolated pathogens 1 week after the final administration of bee venom.

Results

Bacterial identification and results

Table 2 displays the bacterial identification results from milk samples of dairy cows with clinical mastitis. The predominant causative agent was *E. coli* (23 cases, 41.8%), followed by *Serratia marcescens* (7 cases, 12.7%), *Citrobacter koseri* (3 cases, 5.5%), *Klebsiella pneumoniae* (2 cases, 3.6%), *Staphylococcus saprophyticus* (2 cases, 3.6%), and *Pseudomonas aeruginosa* (1 case, 1.8%). No bacterial growth was observed in 15 quarters (15/55, 27.3%). Among the 40 cases with identified pathogens, 36 were caused by gram-negative enteric bacteria, and 4 were caused by gram-positive bacteria.

SCC Changes

Fig. 1 depicts the changes in SCC before and after treatment across 47 quarters affected by clinical mastitis. The somatic cell grade increased from 3.3 pre-treatment to 3.7 on

Table 2. The causative agent and incidence rate of mastitis in 51 dairy cows with clinical mastitis

Microorganisms	No. of quarters	Incidence rate (%)
Aerococcus viridans	1	1.8
Citrobacter koseri	3	5.5
Escherichia coli	23	41.8
Klebsiella pneumoniae	2	3.6
Pseudomonas aeruingonosa	1	1.8
Serratia macescens	7	12.7
Staphylococcus saprophyticus	2	3.6
Streptococcus chromogenes	1	1.8
Unidentification	15	27.3
Total	55	100.0

 https://doi.org/10.17555/jvc.2024.41.6.370

day 3, followed by a gradual decline to 1.7 by day 14. Initially, the administration of bee venom triggered an inflammatory response, which elevated the SCC, eventually leading to a steady decline and recovery.

Efficacy of bee venom treatment

Table 3 presents the therapeutic efficacy of bee venom against clinical mastitis. The treatment effect on *E. coli* (20 quarters) was as such: 2 quarters were cured by day 5, and 8 quarters by day 14. One cow was culled on day 10 following the third dose of bee venom due to exacerbating udder swelling, induration, and lethargy. The influence of bee venom on *Serratia marcescens* (7 quarters) resulted in 1 quarter cured by day 5, while the rest showed no improvement during the treatment period. No therapeutic effect was noted in cases of *Citrobacter koseri* and *Klebsiella pneumoniae* throughout the treatment duration.

The therapeutic effect of bee venom on gram-positive bacteria, comprising *Aerococcus viridans* (1 quarter), *Staphylococcus saprophyticus* (2 quarters), and *Streptococcus chromogenes* (1 quarter), was as follows: 3 out of the 4 gram-positive cases were resolved by day 5, and 1 case by day 14.

For 7 cows with clinical mastitis where no causative bacteria were isolated, the therapeutic effects were as follows: 2 quarters resolved by day 7, 2 by day 10, and 2 by day 14. Of the remaining 11 quarters, 3 showed no improvement by day 14, and 2 worsened, necessitating antibiotic intervention. In total, 22 out of 47 cows with clinical mastitis showed a therapeutic response to bee venom treatment by day 14, excluding the 25 cows that showed no response.

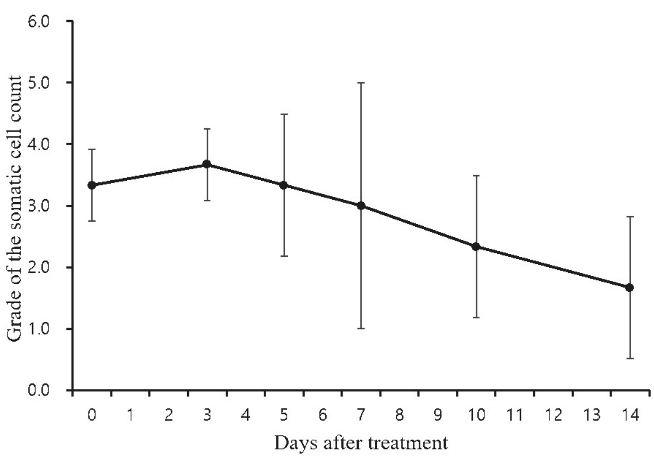

Fig. 1. Changes in somatic cell grade before and after treatment with 12 mg of bee venom once a day for 7 days. The CMT results were converted into grades and expressed numerically. CMT test results are +++ (more than 5 million, 5), ++ (800,000 to 5 million, 4), + (400,000 to 1.5 million, 3), ± (150,000 to 500,000, 2), and – (below 200,000, it is classified into 1).

Discussion

Mastitis is one of the three major diseases affecting dairy cattle and, despite extensive efforts to control it, continues to be a leading cause of economic loss for dairy farmers (33). The costs associated with mastitis are multifaceted, including expenses for pathogen diagnosis, veterinary services, antibiotic use, milk disposal, loss of milk production, premature culling, and even death of the affected cows (18,21,26). Moreover, the use of antibiotics to treat mastitis has serious public health implications, as the emergence of antibiotic-resistant bacteria necessitates the use of stronger antibiotics (33,52). Although numerous reports exist on the treatment of mastitis, a fully realized and established method for its prevention and treatment is still lacking. Antibiotics commonly used to treat mastitis present risks of developing resistance, escalating treatment costs and extending the antibiotic-free

Table 3. **Recovery rate after bee venom treatment in 47 clinical mastitis cases**

Microorganisms	No. of quarters	No. of cured quarters (%)			
		5 day	7 day	10 day	14 day
Aerococcus viridans	1	1 (100.0)	-	-	-
Citrobacter koseri	3	-	-	-	-
Escherichia coli	20	2 (10.0)	-	-	8 (40.0)
Klebsiella pneumonia	1	-	-	-	-
Pseudomonas aeruingonosa	1	-	-	-	1 (100.0)
Serratia macescens	7	2 (28.6)	-	-	-
Staphylococcus saprophyticus	2	1 (50.0)	-	-	1 (50.0)
Streptococcus chromogenes	1	1 (100.0)	-	-	-
Unidentification	11		2 (18.2)	2 (18.2)	2 (18.2)
	47	6 (12.7)	2 (4.3)	2 (4.3)	12 (25.5)

period, thus underscoring the need for alternative therapies.

Bee venom, long utilized in traditional medicine, has recently gained recognition as a natural bioactive substance with potent anti-inflammatory, antibacterial, and analgesic properties. It is also noted for its effectiveness in treating inflammatory diseases and cancer (7,23,25,54). Due to these advantageous effects, bee venom is increasingly used in livestock disease management to prevent and treat diseases and enhance overall productivity (14,54). Previous studies have indicated that bee venom may also be effective in treating mastitis (22). In a study by Kim et al. (27), bee venom proved particularly effective against gram-positive bacteria in clinical mastitis, though it also demonstrated some efficacy against gram-negative bacteria, but with an extended treatment period. Additionally, the response to treatment showed an initial increase in SCC on the third day after bee venom injection, which gradually decreased thereafter. In our study, the SCC was confirmed to increase on the third day after bee venom injection, then steadily declined, falling below 200,000 by day 14 post-treatment. This initial rise could be attributed to an allergic reaction that resulted in the shedding of mammary tissue, followed by the formation of new epithelial tissue. Bee venom contains antimicrobial agents like melittin, apamin, hyaluronidase, histamine, and epinephrine, which likely trigger allergic responses in mammary tissue (9,14).

Bee venom is composed of various bioactive compounds (32), with melittin being the primary substance responsible for its anti-inflammatory and antibacterial effects (37). The composition of melittin in bee venom can vary considerably depending on the season and the region of collection (45), necessitating the standardization of bee venom preparations when considering its use as a natural alternative to antibiotics. Melittin, constituting 40-60% of the dry weight of bee venom, is the key component responsible for its therapeutic efficacy (11,17,39). Studies have indicated that the greater the melittin content, the stronger the therapeutic effects (37). In this study, the bee venom used was sourced from Pakistan, where it consistently showed a melittin content exceeding 58% (Table 1).

The pathogens responsible for mastitis can be categorized into gram-positive and gram-negative bacteria. Gram-negative bacteria, often enteric and environmentally related, contrast with gram-positive pathogens such as *Staphylococcus aureus*, which are typically more infectious. Our findings indicated that environmental enteric bacteria, particularly *E. coli*, were the most prevalent pathogens causing clinical mastitis, aligning with the findings of Xu et al. (52). Of the 40 identified cases of clinical mastitis, 36 were due to gram-negative enteric bacteria, while 4 were attributed to gram-positive pathogens (Table 2). These results underscore the growing challenges in both dairy farming and public health, given the rise in bovine mastitis caused by antibiotic-resistant bacteria (19,37,50,52).

Regarding the therapeutic effects of bee venom, of the 33 cases caused by gram-negative bacteria, 5 were resolved by the 5th day, and 13 showed improvement by the 14th day. However, *Citrobacter koseri* and *Klebsiella pneumoniae* demonstrated no response to the bee venom treatment. In contrast, *Escherichia coli* was cured in 10 out of 20 affected quarters by day 14, resulting in a 50% cure rate. This outcome aligns with previous reports noting the limited therapeutic effect of bee venom on gram-negative bacteria and the need for extended treatment durations (27). These findings emphasize the mounting challenges posed by gram-negative bacteria in dairy farming and public health (52). Further research is required to explore the specific responses of gram-negative bacteria to bee venom treatment and to investigate the potential for combining bee venom with antibiotics.

Recent studies have revealed that many mastitis pathogens are resistant to third-generation antibiotics, an issue intensified by the extended use of antibiotics to treat respiratory diseases and metritis (49). Consequently, there is an increasing effort to develop natural alternatives to replace antibiotics in treating mastitis (13). Our study shows that natural treatments like bee venom can substitute antibiotics, although the treatment duration is longer. Further research is necessary to determine the response of antibiotic-resistant strains to bee venom. In summary, bee venom exhibits a range of therapeutic effects, from no antibacterial activity to effective treatment of mastitis pathogens, and its efficacy varies depending on the bacterial strain, with gram-negative bacteria requiring extended treatment periods.

Acknowledgements

This work was conducted during the research year of Chungbuk National University in 2022.

Conflicts of Interest

The authors have no conflicting interests.

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