

An Exploratory Study of the Potential Antibiotic-Enhancing Effect of Bee Venom Against Uropathogens in Patients with Type 2 Diabetes Mellitus at Brack General Hospital, Libya

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ABSTRACT

Antimicrobial resistance is a growing global health concern, necessitating alternative strategies to improve antibiotic efficacy. This study aimed to conduct an *in vitro* exploratory assessment of the potential antibiotic-enhancing effect of bee venom against uropathogens isolated from patients with type 2 diabetes mellitus at Brack Al-Shati General Hospital, Libya. The study focused on observing preliminary changes in antibiotic susceptibility in the presence of bee venom rather than quantitatively evaluating antibiotic synergy.

Materials and Methods: This laboratory-based exploratory study included 51 patients with T2DM (29 males and 22 females), aged ≥ 40 years, recruited from July 20 to October 1, 2023. Urine samples underwent urinalysis and bacterial culture, followed by isolate identification using conventional methods and the API 20E system. Although 51 patients were enrolled, only nine bacterial isolates were recovered and included in the antimicrobial experiments. Antibiotic susceptibility was assessed using the Kirby-Bauer disk diffusion method, and results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) M100-S27, 27th edition (2017), using species- and antibiotic-specific interpretive criteria. A volume of 50 μ L of undiluted bee venom preparation was applied to each antibiotic disk, and changes in inhibition-zone diameters were assessed descriptively. The Wilcoxon signed-rank test was used for paired comparisons, with emphasis on effect sizes given the small number of isolates. Quantitative synergy assays were not performed.

Results: Nine bacterial isolates were recovered, comprising five Gram-negative and four Gram-positive isolates. *Escherichia coli* was the predominant Gram-negative isolate, whereas *Staphylococcus epidermidis* was the predominant Gram-positive isolate. Overall comparison of inhibition-zone diameters before and after bee venom addition showed no statistically significant change. Among Gram-negative bacteria, the median inhibition-zone diameter decreased from 30.0 mm to 26.0 mm ($P = 0.276$), with a moderate effect size ($r = 0.445$), whereas the median diameter among Gram-positive bacteria remained unchanged at 16.5 mm ($P = 0.498$), with a moderate effect size ($r = 0.303$). Nevertheless, some antibiotic combinations showed an apparent enhancement of antibacterial activity, particularly when bee venom was combined with cefotaxime and gentamicin against selected Gram-negative isolates, and with levofloxacin, cefotaxime, and vancomycin against selected Gram-positive isolates.

Conclusion: Bee venom may have a potential antibiotic-enhancing effect against selected bacterial strains; however, these preliminary observations require confirmation through larger studies using quantitative antimicrobial assays, such as MIC and MBC, together with specialized synergy testing to determine the nature of the interaction between bee venom and antibiotics.

دراسة استكشافية للتأثير المحتمل لسم النحل في تعزيز فعالية المضادات الحيوية ضد مسببات عدوى المسالك البولية لدى مرضى السكري من النوع الثاني في مستشفى براك العام، ليبيا

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سم النحل
مسببات عدوى المسالك البولية
عدوى المسالك البولية
داء السكري من النوع الثاني
مقاومة مضادات الميكروبات
حساسية المضادات الحيوية.

تعتبر مقاومة مضادات الميكروبات من المشكلات الصحية العالمية المتنامية، مما يستدعي البحث عن استراتيجيات بديلة لتحسين فعالية المضادات الحيوية. هدفت هذه الدراسة إلى إجراء تقييم استكشافي *in vitro* للتأثير المحتمل لسم النحل في تعزيز فعالية المضادات الحيوية ضد مسببات عدوى المسالك البولية المعزولة من مرضى السكري من النوع الثاني في مستشفى براك الشاطئ العام، ليبيا، حيث ركزت الدراسة على رصد التغيرات الأولية في حساسية البكتيريا للمضادات الحيوية عند وجود سم النحل، بدلاً من التقييم الكمي للتأثير بينهما.

المواد والطرق: شملت هذه الدراسة الاستكشافية المخبرية 51 مريضاً مصاباً بداء السكري من النوع الثاني (29 ذكراً و22 أنثى)، بأعمار من 40 سنة فما فوق وتمت الدراسة خلال الفترة من 20 يوليو إلى 1 أكتوبر 2023. خضعت عينات البول للفحص الروتيني وزراعة البكتيريا، تلا ذلك تحديد العزلات باستخدام الطرق التقليدية ونظام API 20E. وعلى الرغم من إشراك 51 مريضاً في الدراسة، تم الحصول على تسع عزلات بكتيرية فقط وأدرجت جميعها في التجارب المتعلقة بالنشاط المضاد للميكروبات. تم تقييم حساسية المضادات الحيوية باستخدام طريقة انتشار الأقراص بطريقة Kirby-Bauer وتفسير النتائج وفقاً لمعيار معهد المعايير السريرية والمخبرية (CLSI) M100-S27، الطبعة السابعة والعشرين (2017)، باستخدام المعايير التفسيرية الخاصة بكل نوع بكتيري ومضاد حيوي. أضيف حجم قدره 50 ميكرو لتر من مستحضر سم النحل غير المخفف إلى كل قرص من أقراص المضادات الحيوية وتم تقييم التغيرات في أقطار مناطق تثبيط النمو بصورة وصفية. كما استُخدم اختبار ويلكوكسون للترتيب للمقارنات بين القياسات المزدوجة، مع التركيز على أحجام التأثير نظراً لصغر عدد العزلات ولم تُجرَ اختبارات كمية لتقييم التأثير.

النتائج: تم الحصول على تسع عزلات بكتيرية، منها خمس عزلات سالبة الغرام وأربع عزلات موجبة الغرام وكانت *Escherichia coli* العزلة السائدة بين البكتيريا سالبة الغرام، في حين كانت *Staphylococcus epidermidis* العزلة السائدة بين البكتيريا موجبة الغرام. أظهرت المقارنة الإجمالية لأقطار مناطق تثبيط النمو قبل إضافة سم النحل وبعدها عدم وجود تغير ذي دلالة إحصائية. فبين البكتيريا سالبة الغرام، انخفض الوسط لقطر منطقة تثبيط النمو من 30.0 مم إلى 26.0 مم ($P = 0.276$). مع حجم تأثير متوسط ($r = 0.445$)، بينما بقي الوسط بين البكتيريا موجبة الغرام دون تغير عند 16.5 مم ($P = 0.498$). مع حجم تأثير متوسط ($r = 0.303$) ومع ذلك، أظهرت بعض تركيبات المضادات الحيوية مع سم النحل زيادة ظاهرة في النشاط المضاد للبكتيريا، ولا سيما عند دمجه مع السيفوتاكسيم والجنتاميسين ضد بعض العزلات سالبة الغرام، ومع الليفوفلوكلوكساسين والسيفوتاكسيم والفانكوميسين ضد بعض العزلات موجبة الغرام.

الاستنتاج: قد يكون لسم النحل تأثير محتمل في تعزيز فعالية المضادات الحيوية ضد بعض السلالات البكتيرية المختارة؛ إلا أن هذه الملاحظات الأولية تحتاج إلى تأكيد من خلال دراسات أكبر تستخدم اختبارات كمية للنشاط المضاد للبكتيريا، مثل MIC و MBC، إلى جانب إجراء اختبارات متخصصة للتأثير لتحديد طبيعة التفاعل بين سم النحل والمضادات الحيوية.

Introduction

In the context of the growing challenges associated with the spread of antibiotic resistance, there is an increasing need to explore strategies capable of enhancing the effectiveness of conventional antibiotics, particularly when combined with natural compounds that exhibit strong biological activity [1,2]. Bee venom (*Apis mellifera* L.) is a rich source of biologically active compounds, particularly melittin and phospholipase A2, and has been reported to exhibit direct antibacterial activity against a range of Gram-positive and Gram-negative bacterial species [3,4]. This direct antibacterial activity is distinct from the potential role of bee venom as an antibiotic-enhancing agent, whereby it may enhance the efficacy of conventional antibiotics. However, this potential synergistic effect remains insufficiently established and requires further investigation to confirm its reproducibility and elucidate the underlying mechanisms [5-7].

These effects may ultimately contribute to improved therapeutic outcomes and reduce the development of bacterial resistance by enhancing antibiotic efficacy and lowering the required therapeutic doses. [3,8,9]. This approach is particularly relevant among patients with diabetes mellitus, who are at increased risk of infections, particularly urinary tract infections (UTIs), compared with individuals without diabetes [10,11]. Age is an important clinical consideration in the classification of diabetes mellitus in adults, particularly when distinguishing between type 1 diabetes, type 2 diabetes, and monogenic forms of diabetes [12]. Moreover, advancing age is an established risk factor for type 2 diabetes mellitus (T2DM), with its prevalence increasing markedly with age, particularly after 40 years. Consequently, this age group

carries a greater burden of T2DM and may have an increased susceptibility to UTIs, further emphasizing the clinical relevance of age in the context of diabetes and UTIs [10,11,13]. Diabetes mellitus is one of the most common chronic diseases, with a marked global increase in its prevalence, particularly in low- and middle-income countries, including Libya [14-16].

Despite advances in the study of the antibacterial properties of bee venom, scientific evidence regarding its role as an antibiotic adjuvant against bacterial isolates causing UTIs in diabetic patients remains limited, particularly in local settings. This highlights a clear research gap that requires further experimental investigation.

Accordingly, this study was primarily designed to evaluate the potential enhancement of selected antibiotic activity by bee venom against bacterial isolates responsible for UTIs in patients with T2DM, without presuming or confirming a synergistic interaction.

Material and methods

This exploration laboratory-based study comprised two main components: a clinical sample-collection component and an *in vitro* microbiological experimental component. Urine samples were collected from 51 participants (29 males and 22 females) with a confirmed diagnosis of T2DM, all aged ≥ 40 years. Participants were recruited at Brack General Hospital, Libya, from July 20 to October 1, 2023. The study was conducted at Brack General Hospital following approval from the Technical College of Medical Technology, Brack, in accordance with the requirements of the Scientific Research Ethics Committee (Ethical Approval No. 6.24.003.NBC).

Exclusion Criteria

The exclusion criteria included individuals who had received

antibiotic therapy within two weeks prior to urine sample collection, as recent antibiotic use may suppress bacterial growth and result in false-negative culture results or reduce the likelihood of pathogen isolation. Pregnant women, patients with chronic kidney disease (CKD), and individuals with immune disorders were also excluded. Patients with CKD and individuals with immune disorders were excluded because these conditions may independently increase susceptibility to UTIs and may influence urinary and microbiological findings, potentially introduce confounding factors when assess susceptibility to infection and identifying causative pathogens among patients with T2DM. In addition, participants with incomplete clinical or laboratory data and individuals classified as having prediabetes rather than confirmed T2DM were excluded.

Urine Specimen Collection

Demographic and clinical data, including age, sex, and urinary symptoms, were collected through structured interviews. Morning midstream urine specimens were collected from participants who attended the hospital in a fasting state for routine blood glucose testing. Urine specimens were collected in sterile containers with a capacity of 50 mL. All urine samples were initially subjected to physical, chemical, and microscopic examination.

At the time of sample collection and urinalysis, participants were classified according to the presence or absence of urinary symptoms and the number of pus cells detected in urine. Urine samples from asymptomatic participants with fewer than 10 pus cells/HPF were considered not significant and were not subjected to bacterial culture. In contrast, urine samples showing more than 10 pus cells/HPF, particularly when accompanied by urinary symptoms, were considered suggestive of a possible urinary tract infection and were subjected to microbiological culture for bacterial isolation and identification.

For samples selected for culture, bacterial culture was initiated immediately after completion of the urine examination. The interval between specimen collection and initiation of processing did not exceed 30 minutes. Urinalysis and bacterial culture were performed according to standard microbiological procedures, with strict adherence to aseptic conditions throughout specimen handling, examination, and inoculation to minimize the risk of contamination. Urine samples were collected from all participants to screen for urinary pathogens among patients with T2DM.

Urine Culture and Interpretation of Bacterial Growth

Midstream urine samples were quantitatively cultured on blood agar and MacConkey agar (Oxoid, UK). Before inoculation, each urine sample was thoroughly mixed to ensure adequate resuspension of microorganisms present in the specimen. A calibrated sterile 1-μL loop was used to inoculate the urine sample onto blood agar for quantitative colony enumeration, while a separate sterile loop was used to streak the sample onto MacConkey agar to facilitate the isolation of Gram-negative bacteria.

The inoculated plates were incubated aerobically at 35–37°C for 24 hours. Following incubation, the colonies were counted, and the bacterial load was expressed as colony-forming units per milliliter (CFU/mL). For cultures inoculated using the calibrated 1-μL loop, the bacterial concentration was calculated using the following formula:

$$\text{CFU/mL} = \text{Number of colonies} \times 1,000$$

Bacterial growth was interpreted in conjunction with the presence or absence of urinary symptoms and the results of physical and chemical urinalysis, including urine pH, nitrite, and leukocyte esterase, in addition to microscopic examination findings, particularly the presence and number of pus cells and bacteria, to reduce the likelihood of misinterpreting bacterial colonization or specimen contamination as a confirmed urinary tract infection.

The initial identification of bacterial isolates was based on colony morphology and Gram-stain characteristics. Gram-positive bacteria were identified using catalase and coagulase tests, together with their growth characteristics on Mannitol Salt Agar (Oxoid, UK). All Gram-negative isolates were identified using the API 20E system (bioMérieux, France), and the resulting biochemical profiles were interpreted using the APIweb™ database (bioMérieux, France). Following identification with the API 20E system, an oxidase test was performed as an additional test to assist in confirming and differentiating selected Gram-negative isolates, particularly those belonging to the genus *Pseudomonas*.

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar (Oxoid, UK). Commercial antibiotic disks (Oxoid, UK) were used, and the diameters of the inhibition zones were measured in millimeters (mm). The results were interpreted according to the CLSI Performance Standards for Antimicrobial Susceptibility Testing, 27th edition (M100S, 2017), using the applicable species- and antibiotic-specific interpretive breakpoints.

Bacterial isolates were classified according to the species- and antibiotic-specific interpretive breakpoints specified by the applicable standard. When CLSI breakpoints were applied, isolates were categorized as Sensitive (S), intermediate (I) or resistant (R), where applicable.

Vancomycin was included among the tested antibiotics for exploratory purposes to assess whether its combination with bee venom might result in increased antibacterial activity. It was not used as a clinical susceptibility test for determining the therapeutic susceptibility status of the bacterial isolates.

Definition and Assessment of the Potential Antibiotic-Enhancing Effect of Bee Venom.

In this study, the term “potential antibiotic-enhancing effect” refers to a descriptive increase in the inhibition-zone diameter observed when an antibiotic was tested in combination with bee venom compared with the antibiotic alone.

The inhibition-zone diameter for each antibiotic alone was first measured and interpreted according to the applicable CLSI breakpoints. Following the addition of bee venom, the inhibition-zone diameter was measured again and compared with that obtained using the antibiotic alone. The response was recorded descriptively as an increase, decrease or no change in the inhibition-zone diameter, as illustrated in Figure 1.

No predefined quantitative threshold was established for classifying an increase in inhibition-zone diameter as an enhancing effect. Therefore, the observed changes were considered preliminary descriptive findings and were not interpreted as definitive evidence of synergism. Quantitative interaction analyses, including the checkerboard assay, minimum inhibitory concentration (MIC) determination, and fractional inhibitory concentration index (FICI) calculation, were not performed in this study.

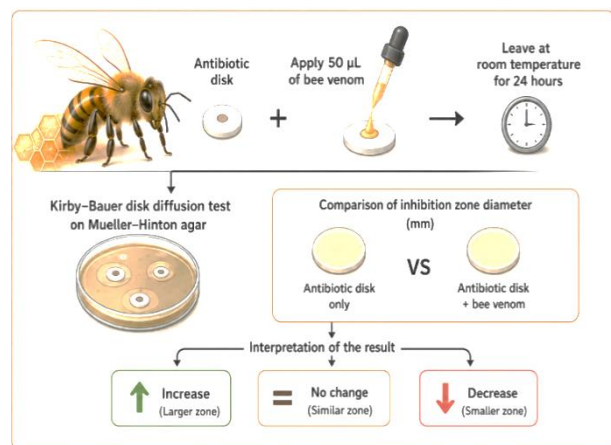


Figure 1: Bee Venom Enhancement of Antibiotic Activity: Kirby-Bauer Disk Diffusion Assay.

Bee Venom Source, Handling, and Preparation for Antibacterial Testing

Raw bee venom was obtained from a local beekeeper in the Al Jabal Al Akhdar region of Libya through a local trader in Sabha. The bee venom was supplied as a liquid preparation in a sterile tube with a total volume of approximately 2 mL. The tube was similar in appearance to a blood collection tube. The preparation did not carry commercial label or manufacturer information, and no data regarding standardized bee venom concentration were available.

The preparation was transported to the laboratory wrapped in paper and protected from direct light exposure. Upon receipt, it was stored under refrigerated conditions in a refrigerator at a temperature ranging from 2 to 5°C until use. The bee venom was used as supplied, without dilution or any further modification. Because the concentration of the received preparation was unknown, the exact concentration of bee venom or its active components could not be determined.

In addition, the concentrations of the major active components of bee venom, including melittin and phospholipase A2 (PLA2), were not quantified or standardized in the preparation used in this study. Therefore, the biological activity of the tested preparation cannot be attributed to a defined concentration of any specific venom component.

A volume of 50 µL of the raw bee venom preparation was applied to each sterile filter-paper disk and to each antibiotic disk. The disks were then left at room temperature for 24 h before being used in the antibacterial activity assay. This step was intended to allow sufficient contact between the bee venom and the antibiotic on the disk before performing the antibacterial activity test. The effect of the 24-h pre-incubation period itself on the biological activity of the bee venom components was not independently evaluated.

Statistical Analysis

Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS), version 27, and Microsoft Excel, version 16. Data distribution was assessed, and continuous variables were expressed as median and interquartile range (IQR) due to the non-normal distribution of the data.

The bacterial isolate was considered the primary unit of analysis, with each antibiotic tested against the same isolate before and after the addition of bee venom constituting a paired comparison. Thus, the inhibition zone diameter measured before bee venom addition was paired with the

corresponding measurement obtained after bee venom addition for the same bacterial isolate and antibiotic. Because individual bacterial isolates could contribute measurements for more than one antibiotic, these antibiotic-specific comparisons were not considered to represent fully independent observations.

The Wilcoxon signed-rank test was used to assess paired differences in inhibition zone diameters before and after the addition of bee venom. Given the very small number of bacterial isolates and the exploratory nature of the study, effect sizes and individual paired changes were emphasized rather than relying primarily on statistical significance based on P-values. The effect size (r) was calculated using the formula $r = |Z|/\sqrt{N}$, where Z represents the standardized test statistic and N represents the total number of paired observations. Effect sizes were interpreted as small ($r = 0.10$ – <0.30), moderate ($r = 0.30$ – <0.50), or large ($r \geq 0.50$) [17]. Individual before-and-after changes were also considered when interpreting the findings. A P-value ≤ 0.05 was considered statistically significant; however, P-values were interpreted cautiously given the limited sample size.

Results

Distribution of Bacterial Isolates

The results showed, based on the limited number of bacterial isolates ($n = 9$), that female participants accounted for the majority of bacterial isolates, with 7 isolates, compared with 2 isolates among male participants. This finding suggests that UTIs were more frequently observed among female participants in the present study. Regarding bacterial distribution, Gram-negative bacteria were more prevalent, accounting for 5 isolates, including *Escherichia coli* with 4 isolates and *Klebsiella spp.* with one isolate. In contrast, Gram-positive bacteria accounted for 4 isolates, comprising *Staphylococcus epidermidis* with 3 isolates and *Staphylococcus aureus* with one isolate.

Antibiotic Activity Against Gram-Negative Bacteria Without Bee Venom (Control Group).

As shown in Table 1, the antibiotic susceptibility patterns of Gram-negative bacterial isolates in the control group (without bee venom) varied among the tested antibiotics. Gentamicin showed complete susceptibility among all tested Gram-negative isolates, with all five isolates (5/5) being sensitive. Cefotaxime and amoxicillin also demonstrated complete susceptibility among the tested isolates, with all available isolates (2/2 for each antibiotic) being sensitive.

In contrast, levofloxacin showed susceptibility in only two of the five tested isolates (2/5), while the remaining three isolates were resistant. Vancomycin showed no susceptibility to the tested Gram-negative isolates, as all tested isolates were resistant. Ampicillin demonstrated limited activity, with most tested isolates being resistant and only one isolate exhibiting intermediate susceptibility.

Table 1: Antibiotic Susceptibility Patterns of Gram-negative

Bacterial Isolates Antibiotic	Number Of Isolates (N)	Sensitive (N)	Intermediate (N)	Resistant (N)
Gentamicin	5	5	0	0
Levofloxacin	5	2	0	3
Vancomycin	5	0	0	5
Cefotaxime	2	2	0	0
Ampicillin	5	0	1	4
Amoxicillin	2	2	0	0

Antibiotic Activity Against Gram- Positive Bacteria Without Bee Venom (Control Group).

As shown in Table 2, Gram-positive bacterial isolates exhibited variable antibiotic susceptibility patterns among the tested antibiotics. Gentamicin demonstrated complete activity against the tested isolates, with all isolates showing sensitivity (2/2). Levofloxacin also showed complete activity, with all tested isolates being sensitive (3/3).

Vancomycin and cefotaxime demonstrated activity against the majority of tested isolates, with sensitivity observed in (2/3) isolates for each antibiotic, while one isolate showed resistance. Amoxicillin exhibited variable activity, with one sensitive and one resistant isolate (1/2). Ampicillin showed no activity against the tested isolate, which was classified as resistant (1/1).

Table 2. Antibiotic Susceptibility Patterns of Gram-Positive

Bacterial Isolates Antibiotic	Number Of Isolates (N)	Sensitive (N)	Intermediate (N)	Resistant (N)
Gentamicin	2	2	0	0
Levofloxacin	3	3	0	0
Vancomycin	3	2	0	1
Cefotaxime	3	2	0	1
Ampicillin	1	0	0	1
Amoxicillin	2	1	0	1

Results for *Ampicillin* should be interpreted cautiously because only one isolate was tested.

Potential Enhancing Effect of Bee Venom on Antibiotic Activity Against Gram-Negative Bacteria.

Table 3 demonstrates variable effects of bee venom addition on antibiotic activity against Gram-negative bacterial isolates. Crude bee venom alone showed limited antibacterial activity. However, when combined with selected antibiotics,

enhancement of antibacterial activity was observed, particularly with cefotaxime and gentamicin. In contrast, ampicillin and amoxicillin showed no improvement following bee venom addition, while levofloxacin and vancomycin demonstrated variable responses among the tested isolates.

Potential Antibiotic-Enhancing Effect of Bee Venom Against Gram-Positive Bacteria.

Table 4 demonstrates variable effects of bee venom addition on antibiotic activity against Gram-positive bacterial isolates. Crude bee venom alone showed limited antibacterial activity. However, enhanced antibacterial activity was observed when bee venom was combined with selected antibiotics, particularly levofloxacin, cefotaxime, and vancomycin. In contrast, gentamicin showed no enhancement following bee venom addition. The observed effect of amoxicillin should be interpreted cautiously due to the limited number of tested isolates.

Potential Antibiotic-Enhancing Effect of Bee Venom on Inhibition Zone Diameters Against Gram-Positive and Gram-Negative Bacteria.

The comparison of inhibition zone diameters before and after bee venom addition showed a slight reduction among Gram-negative bacteria, with the median decreasing from 30.0 mm (IQR = 20.0) to 26.0 mm (IQR = 25.5). This difference was not statistically significant ($P = 0.276$), although the calculated effect size indicated a moderate-to-large effect ($r = 0.445$). Similarly, no change in the median inhibition zone diameter was observed among Gram-positive bacteria, remaining at 16.5 mm before and after bee venom addition, with minor variations in the interquartile range (20.8 vs. 22.4). The difference was not statistically significant ($P = 0.498$), with a moderate effect size ($r = 0.303$) (Table 5).

Table 3: Potential Antibiotic-Enhancing Effect of Bee Venom Combined with Different Antibiotics Against Gram-Negative.

Bacterial Isolates Antibiotic + Bee venom	Number of isolates (n)	Enhanced effect (n)	Reduced effect (n)	No effect (n)
Crude bee venom	5	1	0	4
Gentamicin (GEN) 10 µg	3	2	0	1
Levofloxacin (LEV) 5 µg	5	1	2	2
Vancomycin (VAN) 5 µg	2	1	1	0
Cefotaxime (CTX) 30 µg	4	3	0	1
Ampicillin (AMP) 10 µg	2	0	2	0
Amoxicillin (AMX) 25 µg	2	0	2	0

Table 4: Potential Antibiotic-Enhancing Effect of Bee Venom Combined with Different Antibiotics Against Gram-Positive.

Bacterial Isolates Antibiotic + Bee venom	Number of isolates (n)	Enhanced effect (n)	Reduced effect (n)	No effect (n)
Crude bee venom	4	1	0	3
Gentamicin (GEN) 10 µg	2	0	2	0
Levofloxacin (LEV) 5 µg	2	2	0	0
Vancomycin (VAN) 5 µg	4	3	1	0
Cefotaxime (CTX) 30 µg	2	2	0	0
Amoxicillin (AMX) 25 µg	1	1	0	0

Results for amoxicillin should be interpreted cautiously because only one isolate was tested.

Table 5: Comparison of inhibition zone diameters before and after bee venom addition against Gram-positive and Gram-negative bacteria using the Wilcoxon Signed-Rank Test.

Bacterial group	Number of antibiotics (N)	Before bee venom(mm) Median (IQR)	After bee venom(mm) Median (IQR)	P-value	Effect size (r)	Interpretation
Gram-Negative	6	30.0(20)	26.0(25.5)	0.276	0.445	Moderate
Gram-Positive	5	16.5(20.8)	16.5(22.4)	0.498	0.303	Moderate

Potential Antibiotic-Enhancing Effect of Bee Venom on Antibiotic Response in Identified Bacterial Isolates.

The potential antibiotic-enhancing effects of bee venom supplementation in Table 6. Regarding the effect of bee

venom supplementation on antibiotic susceptibility, *Escherichia coli* isolates exhibited a variable response. Enhanced susceptibility was observed with cefotaxime and gentamicin, whereas resistance or reduced susceptibility persisted with amoxicillin, ampicillin, and levofloxacin. In contrast, *Klebsiella* spp. showed improved susceptibility

when combined with levofloxacin.

For Gram-positive bacteria, both *Staphylococcus epidermidis* and *Staphylococcus aureus* demonstrated increased antibiotic susceptibility following the addition of bee venom, particularly in combination with cefotaxime, vancomycin, and levofloxacin.

Table 6: Potential Antibiotic-Enhancing Effect of Bee Venom Addition on the Antibiotic Activity of Identified Bacterial Isolates.

Bacterial Type	Bacterial Isolate	Antibiotic After Bee Venom Addition	Observed Effect on Susceptibility
Gram-negative	<i>Escherichia coli</i>	Amoxicillin	Persistent resistance with limited improvement observed
Gram-negative	<i>Escherichia coli</i>	Ampicillin	Reduced susceptibility with increased resistance
Gram-negative	<i>Escherichia coli</i>	Cefotaxime	Observed improvement in susceptibility following bee venom addition
Gram-negative	<i>Escherichia coli</i>	Levofloxacin	Reduced susceptibility with persistence of resistant isolates
Gram-negative	<i>Escherichia coli</i>	Gentamicin	Observed enhancement in bacterial susceptibility
Gram-negative	<i>Klebsiella</i> spp.	Levofloxacin	Observed improvement in susceptibility
Gram-positive	<i>Staphylococcus aureus</i>	Levofloxacin	Observed improvement in susceptibility
Gram-positive	<i>Staphylococcus epidermidis</i>	Cefotaxime	Observed improvement in susceptibility
Gram-positive	<i>Staphylococcus epidermidis</i>	Vancomycin	Susceptibility with antimicrobial activity observed
Gram-positive	<i>Staphylococcus epidermidis</i>	Levofloxacin	potential antibiotic-enhancing effect

Discussion

The findings of the present study demonstrated a potential selective enhancing effect of bee venom on the activity of certain antibiotics against bacterial isolates responsible for UTIs among diabetic patients, whereas its direct antibacterial activity when used alone was limited. However, it should be noted that these findings are based on qualitative patterns obtained from the disk-diffusion assay and cannot be considered sufficient evidence of quantitative synergy between bee venom and antibiotics. These findings are consistent with previous studies suggesting that bee venom may serve as a potential biological adjuvant to improve antibiotic efficacy, particularly in the context of increasing antibiotic resistance rates among diabetic patients, who are more susceptible to recurrent infections [2,11,18].

In the current study, crude bee venom alone exhibited no significant inhibitory effect against most bacterial isolates, indicating limited direct antibacterial activity under the experimental conditions applied. However, the observed improvement in the antibacterial activity of some antibiotics following combination with bee venom may be attributed to the presence of biologically active components, particularly melittin. Melittin has been reported to disrupt bacterial cell membranes, increase membrane permeability, and potentially facilitate antibiotic penetration into bacterial cells, thereby enhancing antimicrobial activity [18,19].

The findings of the present study are partially consistent with previous studies reporting that crude bee venom possesses antibacterial activity against several clinically important bacterial species. A recent study demonstrated antibacterial activity of bee venom against *Escherichia coli*, *Salmonella Typhimurium*, and *Enterococcus faecalis*, as evidenced by measurable inhibition zones and relatively low minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values [20]. These findings support the potential of bee venom to exert a direct antibacterial effect. Similarly, the present study demonstrated a potential antibacterial effect of bee venom; however, this effect was limited when bee venom was used alone, whereas a more pronounced enhancement of antibacterial activity was observed when it was combined with certain antibiotics, particularly cefotaxime and gentamicin [21].

Differences in the magnitude of antibacterial activity observed among studies may be attributable to variations in

the concentration of bee venom used, the methods employed to assess antimicrobial activity, the bacterial species or clinical isolates tested, as well as differences in venom composition and geographical or biological origin. Therefore, the findings of the present study do not necessarily contradict previous reports but rather suggest that the antibacterial activity of bee venom may vary depending on the experimental conditions. Moreover, the present findings indicate that its potential role in enhancing the activity of selected antibiotics may be more evident than its direct antibacterial effect when used alone.

In another previous study, bee venom was reported to enhance the activity of antibiotics, with this effect being more pronounced against Gram-positive bacteria than against Gram-negative bacteria [22]. This difference may be partly related to structural differences in the bacterial cell envelope. Gram-negative bacteria possess an additional outer membrane that can act as a protective barrier, potentially limiting the penetration of certain antimicrobial compounds and reducing their access to intracellular targets. In contrast, Gram-positive bacteria lack this outer membrane and may therefore be more susceptible to agents that interfere with cell membrane integrity. Furthermore, the predominance of Gram-negative isolates in the present study is consistent with previous reports indicating that Gram-negative bacteria constitute the major causative agents of UTIs, particularly among individuals with diabetes and those with impaired host defenses [22-25].

The higher prevalence of urinary infections among female participants in the present study may be attributed to anatomical and physiological factors, including the shorter urethral length, proximity of the urethral opening to the anal region, and hormonal changes. These factors increase susceptibility to bacterial colonization and facilitate the development of UTIs [26,27].

Furthermore, a relative improvement in the activity of certain antibiotics, particularly cefotaxime and gentamicin, was observed following their combination with bee venom. This finding suggests that bee venom may enhance antibiotic effectiveness by increasing bacterial membrane permeability or interfering with certain resistance mechanisms, thereby facilitating antibiotic entry and improving antibacterial action [18,28].

No significant improvement was observed when bee venom

was combined with other antibiotics, such as ampicillin and amoxicillin, as most bacterial isolates continued to exhibit high levels of resistance. This observation may be explained by the presence of effective resistance mechanisms, including β -lactamase production or alterations in antibiotic-binding targets, which may reduce the synergistic potential between bee venom and these antibiotics. Moreover, combinations of bee venom with levofloxacin and vancomycin showed variable responses among bacterial isolates, indicating that the antibiotic-enhancing effect of bee venom is dependent on the antibiotic class and bacterial strain rather than representing a universal effect against all bacterial species [4,29].

These findings agree with previous research indicating that the antibiotic-potentiating effect of bee venom is selective rather than generalized. A previous study demonstrated that combining bee venom with gentamicin and vancomycin enhanced their activity against methicillin-resistant *Staphylococcus aureus* (MRSA) isolates, whereas combinations with ampicillin or penicillin showed limited synergistic effects or no clear synergy. These observations support the findings of the present study, in which bee venom enhanced the activity of selected antibiotics, particularly cefotaxime and gentamicin, but showed no significant improvement when combined with ampicillin or amoxicillin. This suggests that the effectiveness of bee venom depends on the interaction between venom components, antibiotic mechanisms of action, and the specific bacterial isolate tested [30].

From a statistical perspective, the overall analysis did not demonstrate statistically significant differences in antibiotic inhibition zone diameters before and after the addition of bee venom among either Gram-negative or Gram-positive bacteria. Although some changes in antibiotic susceptibility were observed following bee venom addition, these changes were not consistent across the two bacterial groups. Moreover, the observed effect sizes suggested varying magnitudes of change at the individual measurement level. Given the limited number of bacterial isolates and available measurements, these findings do not provide sufficient evidence to support a general antibiotic-enhancing effect of bee venom. Nevertheless, the observed patterns may suggest that any potential enhancing effect could be dependent on the bacterial isolate or the antibiotic tested. This interpretation is consistent with previous evidence demonstrating that bee venom and melittin possess direct antimicrobial activity, with greater activity reported against several Gram-positive bacteria than Gram-negative bacteria, suggesting that the antimicrobial effects of bee venom may vary according to the bacterial species and experimental conditions [4,29].

The detailed isolate-based analysis further confirmed this selective activity. *Escherichia coli*, which represented the most frequently recovered isolate in the current study, showed a relative increase in susceptibility following combination treatment with certain antibiotics and bee venom. Similarly, *Klebsiella* spp. exhibited improved susceptibility under the same combined treatment conditions. In contrast, the enhancement of antibiotic activity was more evident among Gram-positive isolates, including *Staphylococcus aureus* and *Staphylococcus epidermidis*, supporting the role of bacterial cell wall structural differences in determining the magnitude and nature of bee venom-mediated antibiotic enhancement. These findings are in agreement with previous scientific reports

[4,18,25,26,28,31,32].

Conclusion

The findings of the present preliminary exploratory study suggest that crude bee venom exhibited limited antibacterial activity when used alone under the experimental conditions employed. However, its combination with certain antibiotics showed a potential and selective antibiotic-enhancing effect against some bacterial isolates, particularly Gram-positive bacteria and selected Gram-negative isolates. These findings suggest that crude bee venom may have potential as an antibiotic adjuvant rather than as a standalone antibacterial agent.

However, the small number of bacterial isolates, the use of crude bee venom, and reliance on the disc diffusion method limit the generalizability of these preliminary observations. Therefore, the findings should be interpreted cautiously and considered hypothesis-generating rather than evidence of confirmed antibiotic synergy or clinical efficacy.

Recommendations

Based on the preliminary findings of this study, future studies are recommended using standardized and, where appropriate, purified bee venom preparations and larger numbers of bacterial isolates to determine whether the observed potential antibiotic-enhancing effect is reproducible. Quantitative antimicrobial assays, including the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), should be used to provide more precise assessment of antibacterial activity and potential interactions between bee venom and antibiotics.

Further investigations are also recommended to explore the potential mechanisms underlying the antibiotic-enhancing effects observed. In addition, appropriately designed vivo studies should be conducted following adequate evaluation of safety and efficacy. Such studies may help determine whether the potential effect observed in the present study is reproducible and clarify the potential therapeutic relevance of bee venom as an antibiotic adjuvant.

Limitations

This study has several limitations. The small sample size and its restriction to a single center limit the generalizability of the findings. Crude, non-standardized bee venom was used without precise quantification of its concentration. Although 50 μ L of the prepared bee venom solution was applied to each disc, the actual volume absorbed by each disc was not measured separately; therefore, the precise amount of bee venom delivered per disc could not be determined. The study relied solely on the disk diffusion method without employing more advanced quantitative assays such as MIC and FICI. In addition, more robust synergy testing methods, such as the checkerboard assay, were not performed. The limited number of isolates in some antibiotic groups, the exploratory nature of the study, and the absence of in vivo experiments and clinical follow-up further restrict the strength and broader applicability of the conclusions.

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