# Comparison of stingless bee honey and silver sulfadiazine on diabetic wound healing in rat models

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**ABSTRACT**

**BACKGROUND** Diabetic foot ulcers (DFUs) are a major health concern in Indonesia. Adjuvant therapies may improve healing by avoiding secondary infections, promoting angiogenesis, and supporting oxygen circulation. This aimed to evaluate the effect of stingless bee honey (SBH) from *Heterotrigona itama* on diabetic wound size in rats (*Rattus norvegicus*), compared to silver sulfadiazine (SSD).

**METHODS** An experimental study was conducted on 13 diabetic wounds in streptozotocin-induced diabetic rats treated with three types of therapies: SSD (n = 5), pure SBH (n = 5), and SBH with 20% water content (n = 3). The study initially involved 21 rats, but eight died during the diabetes modeling and wound observation phases, presumably due to hyperglycemia. Baseline characteristics did not differ significantly across the groups.

**RESULTS** SBH with 20% water content and pure SBH reduced wound size by 95.1% and 92.1%, outperforming SSD (77.4%), with all therapies showing statistically significant improvement ($p<0.05$). However, the differences between groups were not statistically significant ($p = 0.162$).

**CONCLUSIONS** Topically applied SBH is a potential natural therapeutic agent for diabetic wounds, in addition to standard treatment such as SSD.

**KEYWORDS** diabetic foot, honey, silver sulfadiazine, wound healing

Diabetic foot ulcers (DFUs) present a significant global health challenge, often leading to amputation and high mortality rates.[1,2] Importantly, along with diabetic wounds, they are associated with lower-extremity neuropathy, which reduces sensation, making them less sensitive to pressure or thermal injury. Notably, repeated trauma can result in chronic wounds.[3] Wound healing is a complex biological process that involves four phases (hemostasis, inflammation, proliferation, and remodeling) that require coordinated interactions among cellular components, extracellular matrix, cytokines, and growth factors. In diabetic wounds, these phases are often disrupted owing to neuropeptide disorders, hypoxia, and hyperglycemia. Specifically, diabetic wounds cannot control inflammation, angiogenesis,

cytokine expression, or the release of growth factors.[4]

Standard treatments, such as glycemic control, adequate tissue perfusion, wound debridement, off-loading, infection control with appropriate antibiotics, and comorbid management, often yield poor results; therefore, they are combined with adjuvant therapy.[5] Furthermore, wound care using modern moisture-based dressings has significantly improved tissue healing by maintaining moisture balance, improving oxygen exchange, isolating proteases, stimulating growth factors, preventing infection, facilitating autolytic debridement, and promoting granulation tissue production and reepithelialization.[6] Notably, honey, a long-used natural product, can further improve DFU treatment outcomes when combined with standard care.

The trigona bee (*Trigona sp.*) produces stingless bee honey (SBH), traditionally used in Indonesia for its medicinal properties, particularly in Kalimantan. These bees produce honey, pollen, and propolis, which are beneficial to humans.[7] The benefits of honey stem from its biochemical composition and its antioxidant, anti-inflammatory, antibacterial, and moisturizing properties. Specifically, antioxidants can damage free radical chains that harm the injured areas, whereas their anti-inflammatory properties protect tissues from damage caused by toxic inflammatory mediators. Additionally, the hydrogen peroxide ($H_2O_2$) in SBH has antibacterial properties that benefit the wound healing process by causing oxidative damage, inhibiting bacterial growth, and degrading DNA.[8] Notably, SBH produced a higher mean inhibition of common DFU pathogens, such as *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*, compared to white and yellow honey derived from *Apis mellifera*.[9] Its moisturizing properties help prevent secondary infections, trigger angiogenesis, and ensure adequate oxygen circulation, thereby candidating SBH as a DFU treatment.

This study aimed to evaluate the effect of SBH (*Heterotrigona itama*) on diabetic wound size in rats, selected for their comparable wound-healing phases and skin structure to humans, despite their thinner epidermis. No prior study has directly compared the wound-healing effects of SBH to those of silver sulfadiazine (SSD), a widely used treatment known to enhance DFU healing through antibacterial mechanisms.[10] Therefore, the present study investigated the therapeutic potential of SBH relative to that of SSD in promoting diabetic wound recovery.

## METHODS

A true experimental laboratory design was established at the Biochemistry and Biomolecular Laboratory, Faculty of Medicine, Universitas Lambung Mangkurat. The research protocol was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Lambung Mangkurat (No. 368/KEPK-FK ULM/EC/X/2023). Initially, 21 healthy male rats (*Rattus norvegicus*; 250–350 g, aged 2–4 months, blood glucose levels of <200 mg/dl) were randomly allocated to three groups of seven rats each using simple randomization. The sample size was calculated using a 95% confidence interval (CI) and 80% study power.[11]

The rats were transferred to new cages for a 1-week acclimation period. Following acclimation, the remaining rats were fasted for 12 hours before diabetes induction. Diabetes was modeled by intraperitoneal injection of streptozotocin (STZ) at 50 mg/kg. After 3 days, blood glucose levels were measured, and diabetes was confirmed in rats with levels ≥200 mg/dl. Treatment began on the third day following wound induction, and routine health monitoring of the rats was performed at least once every 3 days.

Anesthesia was induced via the intramuscular administration of 0.05 cc of ketamine. The dorsal fur was shaved, and wounds were made using a 3.5 mm diameter punch biopsy, scissors, and tweezers. The wounds were left untreated for 2 days before treatment initiation.

Diabetic wounds were treated with SSD (control group), pure SBH (group 1), and 20% water content SBH (group 2). The treatment was administered once daily, and the intervention was consistently administered daily between 9 a.m. and 10 a.m. Wound sizes were measured daily using a digital caliper, with the area calculated by multiplying the maximum length (vertical dimension) and width (horizontal dimension). Measurements were conducted immediately after wound induction and continued until Day-15 following wound treatment initiation.

Statistical analyses were performed using SPSS software version 21.0 (IBM Corp., USA), while data visualization was performed using GraphPad Prism version 10.0 (GraphPad Software Inc., USA). Paired

t-tests assessed within-group differences after confirming a normal distribution. Analysis of variance evaluated the post-therapy differences between the groups. Wound reduction (%) was calculated by subtracting the wound area measured 2 days before treatment (baseline) from the wound area on Day-15, dividing the difference by the baseline wound area, and multiplying the result by 100.

## RESULTS

During the modeling and observation periods, several rats died, presumably because of hyperglycemia. Overall, eight rats were two rats in control, two in group 1, and four in group 2, leaving 13 rats to complete the study. However, baseline characteristics were statistically comparable across groups in the final sample size. The study proceeded with available samples in the control group (n = 5), group 1 (n = 5), and group 2 (n = 3).

**Table 1.** The mean changes of body weight and blood glucose levels on each group before and after acclimation

| Type of therapy | Change in BW (g) | Change in blood glucose levels (mg/dL) |
|---|---|---|
| Silver sulfadiazine, mean (SD) | –87.000 (66.106) | 223.600 (249.995) |
| Pure SBH, mean (SD) | –76.000 (52.726) | 219.800 (222.791) |
| 20% water-content SBH, mean (SD) | –46.667 (77.675) | 291.667 (282.298) |

BW=body weight; SBH=stingless bee honey; SD=standard deviation

Table 1 displays the changes in the health conditions of all rat groups before STZ injection and after the 12$^{th}$ day of therapy. All groups experienced weight loss and elevated blood glucose levels during the study period. The control group exhibited the greatest weight loss, whereas the group treated with 20% water-content SBH demonstrated the lowest weight reduction but the highest blood glucose increase.

Figure 1 illustrates the effects of SSD on the wounds of the diabetic rats. The wounds dried by the 7$^{th}$ day of therapy and continued to shrink until the final observation on the 15$^{th}$ day, although this treatment resulted in slight scarring. Figure 2 displays a marked wound size reduction on the 7$^{th}$ day of therapy. Most wounds treated with pure SBH were almost completely closed. Overall, it was challenging to identify the original wound sites. Figure 3 presents the wounds treated with SBH with 20% moisture, which exhibited a shrinkage pattern similar to that observed for the pure SBH group. One sample showed a reddish wound appearance, indicating a slight variation compared with the other two samples within the same treatment group.

Figure 4 illustrates the wound size changes across groups. An increased wound size was observed in all groups on the first day of therapy. Two days before therapy initiation, wound sizes were the largest in the pure SBH group, followed by the SSD group, and smallest in the SBH with 20% water content group. Over the 15 days after therapy, the wound size remained the largest in the SSD treatment group, followed by the pure SBH and SBH with 20% water content groups. Wound size decreased in all therapy groups, with the

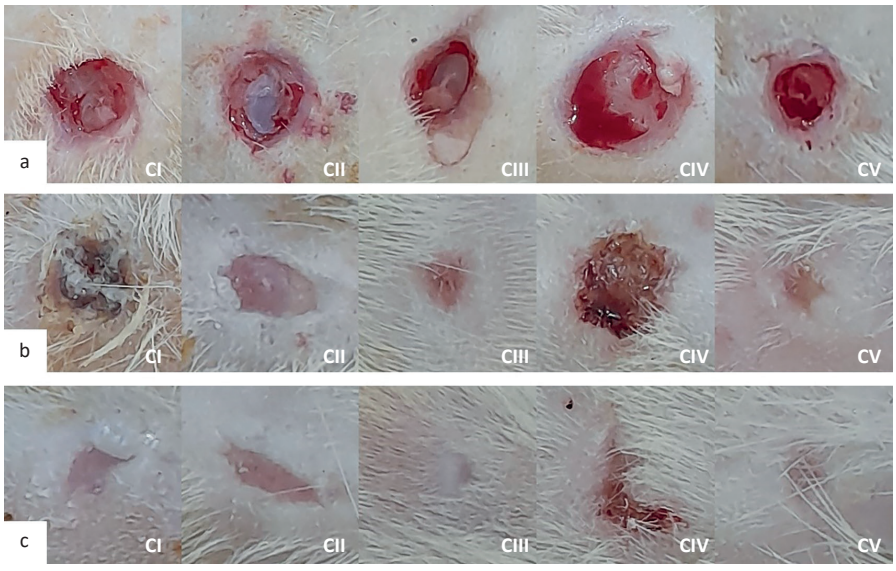


**Figure 1.** The appearance of wounds treated with silver sulfadiazine. (a) Condition of the wound prior to therapy; (b) condition following the 7$^{th}$ therapy; (c) condition following the 15$^{th}$ therapy

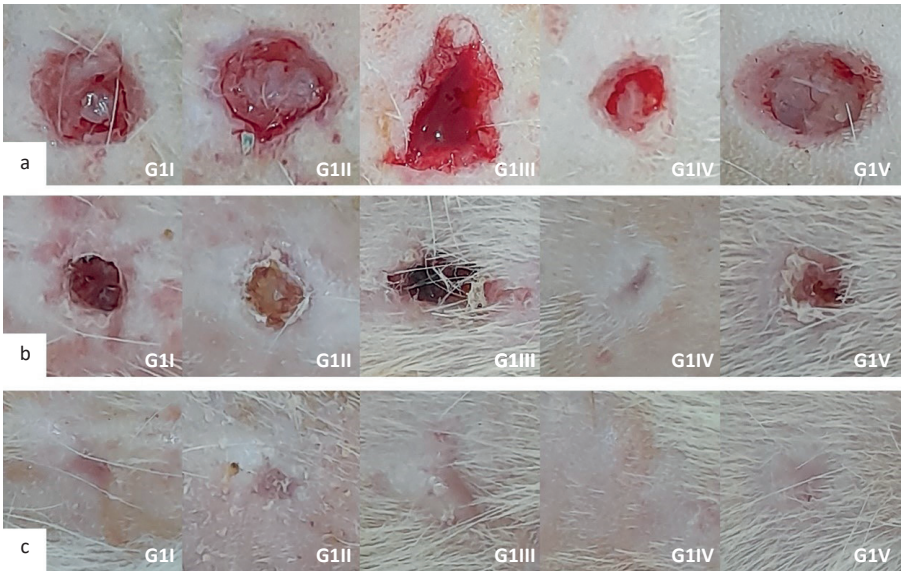


**Figure 2.** The appearance of wounds treated with pure stingless bee honey. (a) Condition of the wound prior to therapy; (b) condition following the 7th therapy; (c) condition following the 15th therapy

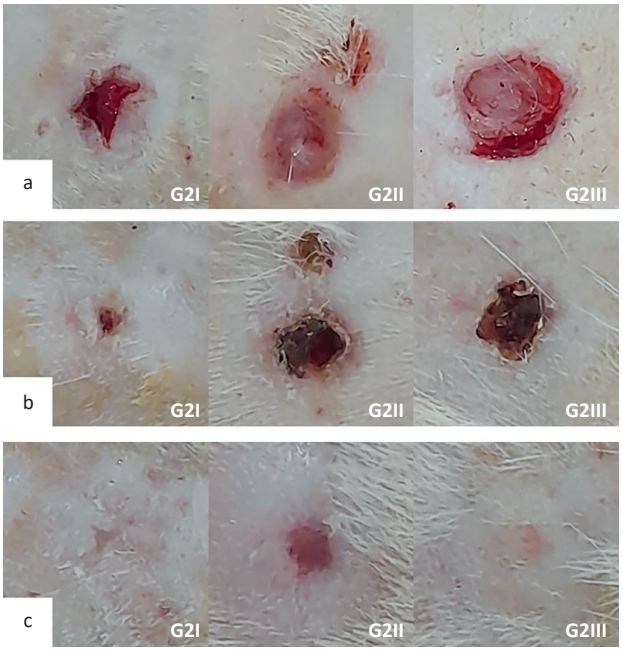


**Figure 3.** The appearance of wounds treated with 20% water-content stingless bee honey. (a) Condition of the wound prior to therapy; (b) condition following the 7th therapy; (c) condition following the 15th therapy

greatest percentage reduction in rats treated with 20% water-content SBH compared with pure SBH and SSD.

Paired *t*-test results implied that all treatments positively affected diabetic wound healing based on wound size reduction ($p<0.05$). Although the pre-post therapy for each group exhibited significant improvements, the opposite result was observed when the wound size after therapy in all groups was tested simultaneously. The differences between the groups were not statistically significant ($p = 0.162$).

## DISCUSSION

Topically applied SBH might accelerate diabetic wound healing, with a greater wound reduction percentage compared to that of SSD. However, literature on SBH use in diabetic wounds remains limited. A similar study compared the effectiveness of SSD using honey variants from different geographical regions of Nigeria, finding that rat wounds gradually reduced to 67 ± 16% after 9 days of SSD therapy. Meanwhile, the honey treatment groups reduced the average wound size of rats to 90 ± 0%, 55 ± 10%, 74 ± 11%, 67 ± 8%, 68 ± 7.5%, and 72 ± 7.5%, providing significant improvements ($p<0.001$).[12]

SBH contains several anti-inflammatory compounds.[13] Its anti-inflammatory effects may be associated with reactive oxygen species inactivation.[14] Moreover, at 50% concentration, it demonstrated the highest mean inhibition across standard and resistant strains of *E. coli*, *S. aureus*, and *K. pneumoniae* (22.27 ± 3.79 mm) compared with white (21.0 ± 2.7 mm) and yellow *A. mellifera* (18.0 ± 2.3 mm) honey at 50% (v/v) concentration.[9] Additionally, SBH from *Heterotrigona itama* inhibited the growth of *S. aureus* and *E. coli* at concentrations as low as 5%[15] because of its $H_2O_2$ content.

A previous study using SBH samples demonstrated that the levels of $H_2O_2$ ranged from a minimum of 111.07 µmol/L to a maximum of 192.82 µmol/L, much higher than those detected in honeydew honey (183.51 µmol/L) and blossom honey (137.61 µmol/L).[16] This SBH property warrants further exploration given

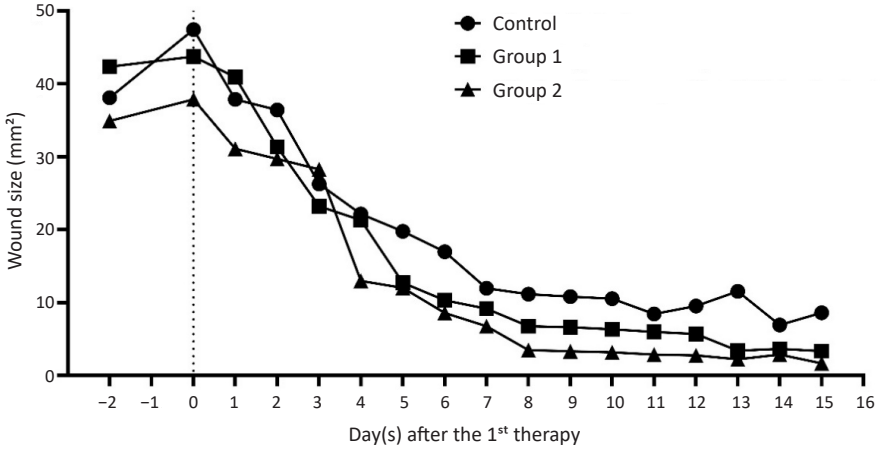


**Figure 4.** Comparison of daily changes in rats' wound size based on the therapy groups. Control=treated with SSD; group 1=pure SBH; group 2=20% water-content SBH. SBH=stingless bee honey; SSD=silver sulfadiazine

the critical role of bacterial diversity in diabetic foot infection prognosis, likely established during the hemostatic phase.[17,18]

Another beneficial SBH component is its high total phenolic content and flavonoid levels, which reach 6.56 ± 0.03 mg GAE/g and 5.80 ± 0.03 mg QE/g, respectively.[15] The SBH phenolic compounds exert antioxidant effects that can prevent free radicals.[19] Moreover, SBH can inhibit interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) expression by 43.9% and 23%, respectively, avoiding persistent inflammation influenced by various cytokines and their expressions, including IL-6 and TNF-α.[18]

Persistent inflammation during the proliferation and remodeling phases can reduce collagen deposition and tensile strength;[20] thus, adequate oxygen circulation is important to support these healing processes.[21] SBH achieves this owing to its naturally high water content. A comparative study found that SBH *Trigona* spp. bees contain 25–31% water, whereas honey from *A. mellifera* bees averages 18.70%.[13] Notably, higher moisture levels in SBH facilitate fermentation and crystallization, potentially reducing its quality.[22] Therefore, reducing the water content of honey by up to 20% is recommended to inhibit microbial growth, which degrades the honey quality and extends its shelf life.[23,24] Accordingly, this study evaluated SBH with a water content reduction of 20%, demonstrating that diabetic wounds treated with 20% water-content SBH exhibited a higher percentage of wound reduction than did the other groups. Importantly, inflammation was observed during the second measurement in this study when wounds were left for 2 days without therapy. During this period, wound size increased in all groups. The following day, the wound shrank slightly after the first therapy.

In this study, diabetes was induced by an intraperitoneal dose of 50 mg/kg STZ, because a lower 40 mg/kg dose was deemed inadequate for establishing a diabetic animal model.[25] Nonetheless, several rat models died from hyperglycemia, as demonstrated by recent routine health examinations, which revealed excessively high blood glucose levels. Glycemic control is crucial for the healing of diabetic wounds.[26,27] A continuous increase in plasma glucose levels causes endothelial cell dysfunction and decreases capillary vasodilation and nitric oxide levels, thereby interfering with tissue perfusion, ischemia, and ulcers while promoting bacterial growth.

Both SBH and SSD are relatively safe for topical application. Direct honey application to wounds may cause a temporary stinging sensation. Importantly, caution is advised when using SSD, particularly in individuals with previous hypersensitivity reactions, pregnant women, and infants younger than 2 months of age.[28,29]

The study had a relatively small sample size, which may have limited the broader applicability of the findings. In addition to the biological and physiological factors of the experimental animals, diabetes induction methods, wound size and location, routine health monitoring, and wound measuring techniques, this study did not control for potential confounding factors such as housing conditions, stress, and glucose control through specialized diets or supplementation. Consequently, these results should be interpreted with caution in subsequent clinical investigations. These methodological constraints must be considered when applying the outcomes to clinical practice.

Nevertheless, this study provided valuable preliminary evidence that advances our current understanding of the efficacy of SBH in DFU management. To enhance external validity, future studies should prioritize larger sample sizes, balanced group allocations, and comparative analyses with additional therapeutic modalities; establish standardized criteria for the honey used; ensure integrity and adherence to established research guidelines; and emphasize glycemic control in experimental animals. Although there are no strict requirements for maintaining balanced sample sizes across trial groups, an equal number of samples can offer statistically better results.

In conclusion, compared to SSD, SBH supports diabetic wound healing in each phase by enhancing recovery and reducing wound size, particularly in SBH with 20% water content. There have been few quantitative studies evaluating the benefits of SBH from *H. itama* for wound healing, particularly experimental studies that directly assess the effect of SBH on the size of diabetic wounds. Therefore, this preliminary study for future research examined the effect of SBH on various biomarkers of diabetic wound healing.

**Conflict of Interest**

The authors affirm no conflict of interest in this study.

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