A Comparative Evaluation of the Wound Healing Properties of Stingless Bee Honey in Diabetic Animal Models

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Abstract

Diabetes is commonly associated with delayed wound healing, highlighting the need for alternative wound management approaches. This study evaluates the wound-healing potential of honey from two stingless bees from Brunei Darussalam, *Geniotrigona thoracica* and *Heterotrigona itama*, in alloxan-induced diabetic Wistar rats. The honey was applied topically at 100% concentration to excision wounds for a period of 14 days, with normal non-diabetic and diabetic untreated rats used as controls. Wound contraction was assessed using ImageJ while histological analysis was performed on Day-6 post wounding to evaluate tissue repair. Wound contraction increased in all groups with *G. thoracica* treated group showing significantly higher wound contraction on Day 7 and Day 10 at 72.9 ± 6.7% and 92.3 ± 2.09 % respectively. Histological analysis on Day 6 showed reduced inflammatory cell infiltration, increased collagen deposition, and improved re-epithelialisation in the honey-treated groups. These findings suggest that Brunei stingless bee honey, particularly *G. thoracica*, may promote wound healing in diabetic rats and has potential as a natural wound management agent.

Index Terms: Geniotrigona thoracica, Heterotrigona itama, stingless bee honey, wound healing, diabetic animal model

1. Introduction

Diabetes mellitus is a chronic metabolic disease caused by chronic hyperglycaemia and is associated with a wide range of systemic complications.[1] This increasing prevalence has contributed to an increased burden of diabetes-related complications, including chronic wounds, infections and delayed tissue repair, which collectively reduce quality of life and increase healthcare costs.[2,3] Among the complications of diabetes, impaired wound healing remains a clinical challenge,[4] particularly because the normal wound healing process is dependent on a coordinated sequence of including haemostasis, inflammation, proliferation, and remodelling.[5] Under normal physiological conditions, these phases occur sequentially to restore the integrity of damaged skin. However in diabetic individuals, this process is often disrupted by prolonged inflammation, impaired angiogenesis, reduced fibroblast migration, diminished collagen deposition, excessive oxidative stress, and weakened immune responses, leading to delayed wound healing, poor tissue regeneration and ultimately increase the risk of infection.[6]

Natural resources have long been used in traditional medicine because they are affordable, accessible, biocompatible and possess a variety of biological activities.[7] Due to limitations in conventional wound treatments, including high cost, low efficiency, and allergy side effects, attention has been directed towards substitutes like natural products.[8] Various natural products including plant extracts, fungi and animal products have been investigated for their wound

healing potential.[9,10] Among these, honey has gained significant interest because of its traditional medicinal use and wound-healing effects.[11] The therapeutic potential of honey is associated with its complex composition of carbohydrates, proteins, enzymes, vitamins, amino acids, phenolic acids, flavonoids, antioxidants and essential minerals.[12] These bioactives contribute to the potential application of honey in wound management. Furthermore, honey may support wound healing through its low pH, high osmolarity and rich phytochemical profile while also functioning as a natural moisturiser that maintains an optimal wound environment for granulation tissue formation and re-epithelialization.[13]

Stingless bee honey, also known as meliponine honey, is widely distributed in tropical areas and is known to possess stronger medicinal properties than conventional honey.[14,15] The two commonly cultivated stingless bee species in Southeast Asia are *Heterotrigona itama* and *Geniotrigona thoracica*. Stingless bee honey is highly valued for its antioxidant content and rich phenolics and flavonoids including as p-coumaric acid, salicylic acid, caffeic acid, ferulic acids, pinocembrin, chrysin, galangin and quercetin.[16] Moreover, stingless bees store their honey in cerumen pots composed of beeswax and plant resins, enriching the honey with additional phytochemicals.[17,18] Phenolic acids and flavonoids act as antioxidants by scavenging reactive oxygen species (ROS), thereby protecting fibroblasts and promoting collagen synthesis and extracellular matrix formation, essential for wound contraction.[19,20] Additionally these compounds also exhibit anti-inflammatory effects by reducing proinflammatory cytokines and improving the transition from the inflammatory to the proliferative phase of wound healing. Studies have shown improved epithelialization and wound bed quality in diabetic patients treated with stingless bee honey-based dressings.[20-22]

Despite the reported properties of stingless bee honey, comparative studies between *H. itama* and *G. thoracica* in diabetic animal models remain limited. Therefore, this study aimed to evaluate and compare the wound-healing potential of both honey in alloxan-induced diabetic Wistar rats.

2. Experimental methods

2.1 Honey collection and preparation

The stingless bee honey of *H. itama* and *G. thoracica* were collected from Tasbee Meliponiculture Farm, Sungai Kelugos in Tutong District, Brunei Darussalam (4°49′02.0″N, 114°45′48.2″E), on September 27, 2025. Honey samples were collected directly from beehives using a sterile syringe. The treatments consisted of 100% pure stingless bee honey from *H. itama* and *G. thoracica* bees. Honey was applied topically to the wounds immediately after wound formation, without dilution or incorporation of any carrier substances.

2.2 Animal experimental work

Eight-week-old male Wistar rats weighing more than 250 g were used in this study. A total of 24 rats were randomly assigned into four groups, with six rats per group (n = 6). Each rat underwent a single excision wound on the dorsal thoracic region, as rats are commonly used in wound-healing studies.[23] The animals received topical treatment daily for a duration of 14 days.

The experimental groups were classified as follows:
Group 1: Normal control - non-diabetic wounded rats with no treatment.
Group 2: Diabetic control - diabetic wounded rats with no treatment.
Group 3: *H. itama* honey treatment - diabetic wounded rats treated with 100% *H. itama* honey.
Group 4: *G. thoracica* honey treatment - diabetic wounded rats treated with 100% *G. thoracica* honey.

2.2.1. Induction of diabetes and excision wound model

Alloxan monohydrate (Sigma-Aldrich, United Kingdom) was used to induce experimental diabetes in Wistar rats.[24] The animals in this study for groups 2, 3, and 4 were induced to develop diabetes. 12 hours prior to the alloxan injection, the rats were fasted but had unlimited

access to water *ad libitum*. The dose administered for each intraperitoneal (IP) injection was 180 mg/kg body weight for each rat.[25] The required injection volume was calculated individually for each animal using the formula:

$$Injection\ volume\ (mL) = \frac{Dose\ (mg/kg)\ \times Body\ weight\ (g)}{Solution\ concentration\ (mg/mL)}$$

After 72 h, their blood glucose levels was measured using a glucometer. Animals with a blood glucose reading of 11.1 mmol/L (200 mg/dl) and above were considered diabetic.[26] For the excision procedure, the rats were individually anesthetised using diethyl ether prior to wound excision. The depth of anesthesia was assessed by observing the rats' reflex responses, such as spontaneous movements, tail and limb extension in response to stimuli.[27] Once deep anesthesia was confirmed, the wound excision was carried out immediately where skin was carefully excised using forceps and surgical scissors. The rats were then returned to their individual cages to recover for a day before the wound assessment.

2.2.2. Wound contraction assessment

Topical application of prepared ointments on the wound area was applied once daily for a period of 14 days for each treatment group. A ruler was placed near the wound and photographs were taken with a digital phone. The wound area was analyzed using Image J software (National Institute of Health, USA). Furthermore, the percentage wound contraction was calculated from the initial wound area size, according to the following:

$$Percentage\ Wound\ Contraction = \frac{(Inital\ wound\ area\ size\ -Specific\ day\ wound\ area\ size)}{Initial\ wound\ area\ size} \times 100$$

The wound contraction calculation was expressed as a percentage and as an average values.

2.2.3. Histological analysis of skin tissue

Histological analysis was performed on Day 6 post-wounding to assess tissue repair during the proliferative phase of wound healing. One rat from each group was euthanised and skin tissue fragments measuring approximately 0.5–1.0 cm² were excised from the wound region. The tissue samples were fixed in 10% neutral buffered formalin for 24–36 h, followed by standard processing.

The fixed tissues were dehydrated using ethanol and subsequently cleared in xylene, embedded in paraffin wax, and sectioned at approximately 5 µm thickness using a rotary microtome. The sections were mounted onto microscope slides and stained with haematoxylin and eosin (H&E) to evaluate general tissue morphology. Histological analysis was conducted from the tissues of animals at Day 6 post-wounding.

2.3 Statistical analysis

The data for wound contraction percentage were expressed as mean ± SEM. Statistical analyses were conducted with one-way Analysis of Variance (ANOVA) and a post hoc Tukey's HSD test on the wound contraction. These were performed using R 4.2.1 software.

3. Results

The wound images shown in ***Figure 1*** were randomly selected from animals representing the individual experimental groups. On Day 3 post-wounding, noticeable changes were observed across all groups, with most groups displaying scab formation. On Day 7 post-wounding, wound sizes decreased as the scab crust thickened in all groups. The most noticeable reduction in wound size was observed in the normal control and *G. thoracica* treated groups. On Day 10 post-wounding, the scab continued to thicken at the wound surface in the diabetic control, *G. thoracica* and *H. itama* treated groups. However, in the normal control group, the scab had likely detached from the wound surface, revealing a new, thinner, reddish scar. All groups showed a marked difference in wound size compared to their initial wounds, although the diabetic control group had the largest wound size. On Day 14 post-wounding, the normal control and *H. itama* treated groups achieved complete wound closure, with white, healed skin observed across all groups. However, the diabetic control and *G. thoracica* treated groups showed delayed healing

as a small crust and pinkish skin were still observed at the wound site (see ***Figure 1***).

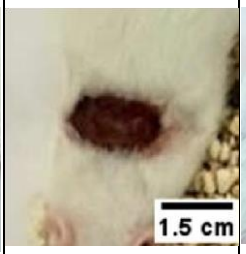
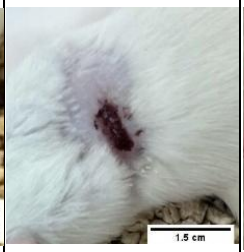
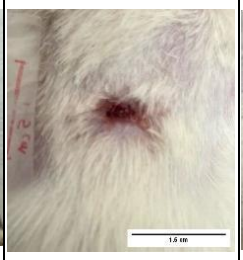
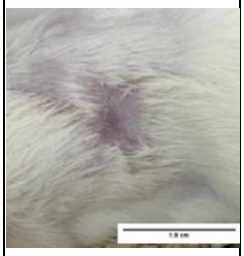
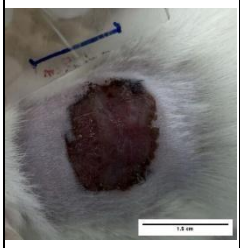
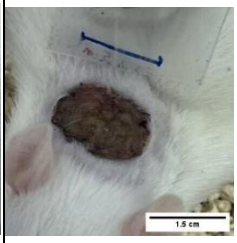
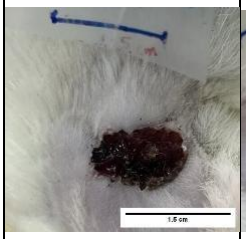
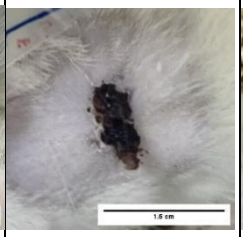
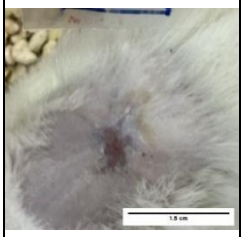
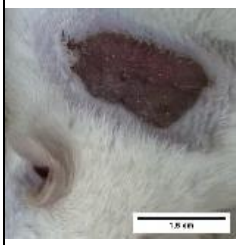
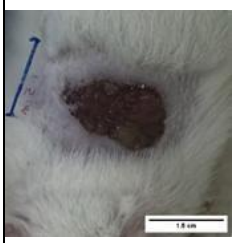
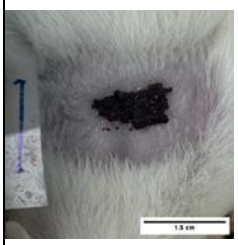
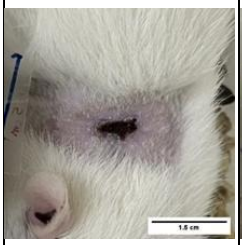
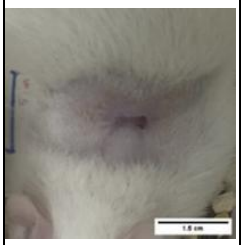
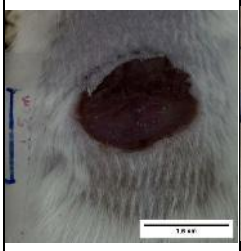
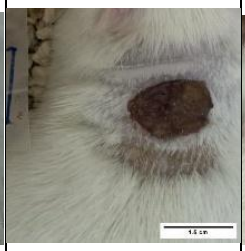
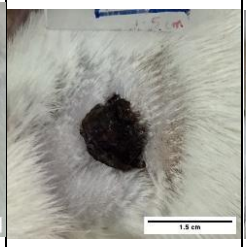
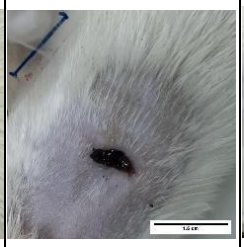
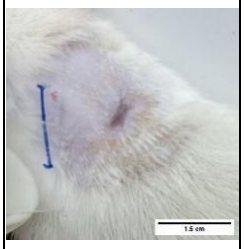
	Day 0	**Day 3**	**Day 7**	**Day 10**	**Day 14**
Normal Control					
Diabetic Control					
G. thoracica					
H. itama					

Figure 1. Macroscopic observation of wound healing progress in normal non-diabetic control, diabetic control and diabetic treated groups at given post-wounding time days (Days 0, 3, 7, 10, and 14). The scale bar represents 1.5cm.

On Day 6 post-wounding, distinct differences in tissue structure and cellular response were observed across the four groups. A scab was observed in the diabetic control group wound

(see ***Figure 2C***), whereas in the *H. itama*-treated group, the epidermal layer showed reattachment (see ***Figure 2E***). A thick epidermal layer was observed in the normal control and diabetic honey-treated groups (see ***Figures 2A***, ***2E*** and ***2G***). The diabetic control group exhibited impaired healing, characterised by reduced cellular density and disorganised tissue structure (see ***Figures 2C*** and ***2D***). In contrast, the normal control group showed a typical proliferative response, with well-organised granulation tissue, dense cellular infiltration, abundant fibroblast proliferation, early matrix organisation (see ***Figures 2A*** and ***2B***). Treatment with stingless bee honey improved the histological features of wound healing in diabetic rats. The diabetic + *H. itama* treated group demonstrated improved tissue organisation showing continuous epithelial layer with increased fibroblast distribution within the dermis. The overall tissue structure showed improved alignment and reduced wound gaps (see ***Figures 2E*** and ***2F***). The diabetic + *G. thoracica* treated group showed moderate improvement compared with the untreated diabetic control group. Collagen deposition and vascular structures were present but less prominent compared with the *H. itama*-treated group. Some residual tissue gaps were still visible within the wound area (see ***Figures 2G*** and ***2H***). Overall, the normal control group demonstrated the most organised tissue structure, while the diabetic control group showed the most disrupted histology. Both honey-treated groups had improved histological features.

Table 1 shows that wound contraction increased progressively over time in all groups. On Day 3 post-wounding, all groups except the non-diabetic control group showed more than 20% wound contraction. The non-diabetic control group showed significantly lower wound contraction (0.42 ± 4.24%) than the diabetic control group (20.11 ± 3.94%) and the honey-treated groups, *H. itama* (27.97 ± 3.06%) and *G. thoracica* (24.36 ± 7.22%).

On Day 7 post-wounding, wound contraction continued to improve in all groups. *G. thoracica* showed the highest percentage of wound contraction at 72.9 ± 6.7 % (see ***Table 1***). No significant differences were observed among most groups but the diabetic control group showed significantly lower wound contraction than the *G. thoracica treated group (Table 1).*

At Day 10 post-wounding, the diabetic control group showed significantly lower wound contraction (%) values compared to the other groups (see ***Table 1***). Both *G. thoracica* group (92.3 ± 2.09 %) and *H. itama* (90.9 ± 3.34 %) treated groups were not significantly different from the non-diabetic control group. These three groups had significantly higher wound contraction values than the diabetic control group (82.6 ± 5.03 %).

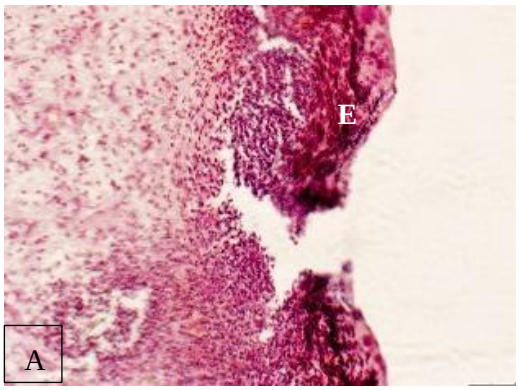
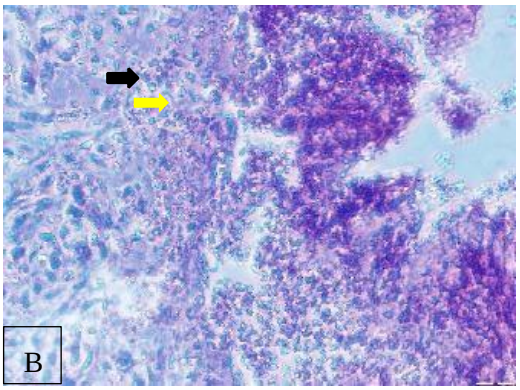
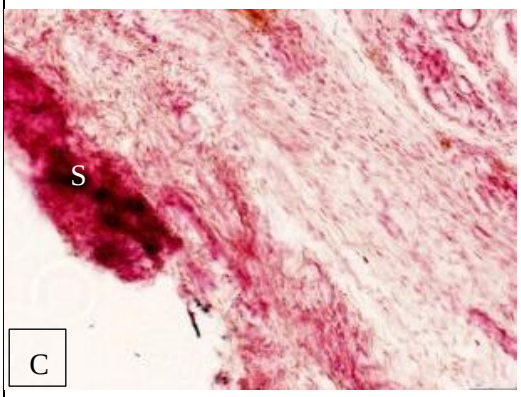
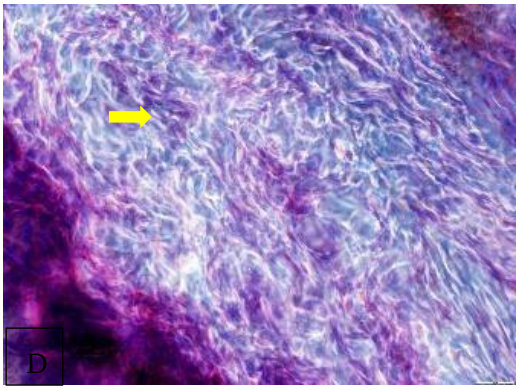
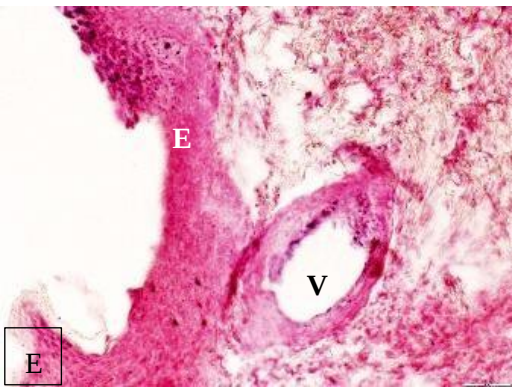
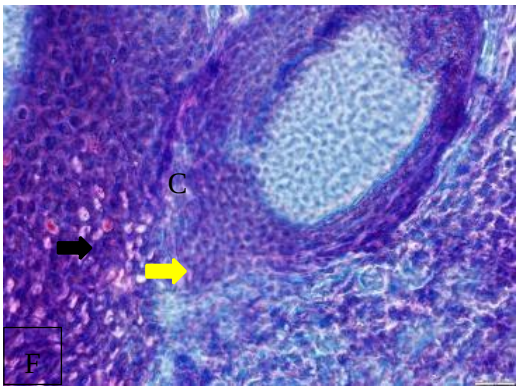
By Day 14 post-wounding, wound contraction continued to increase in all groups, with all groups approaching near-complete wound closure (see ***Table 1***) and no significant differences were observed among the groups. The raw data of the wound contraction of each animal is shown in ***Supplementary Tables 1-3***.

4. Discussion

The wound healing process is a multi-factor biological process, that can become dysfunctional in diabetes, resulting in delayed healing and persistent wounds.[28] Diabetes can interrupt the healing process and increase the probability of infection in the wounded area, leading to prolonged hospitalization.[29] This may be due to increased oxidative stress and wound infection, impaired leucocyte functions, and inadequate migration of neutrophils neutrophils.[30] In addition, the accumulation of advanced glycation and end products in diabetic skin makes the skin more susceptible to infection.[31] Alloxan-induced diabetic models mimic type 1 diabetes mellitus (T1DM) by causing oxidative stress that destroys pancreatic β-cells, making them a valuable model for studying diabetic wound healing.[32]

In this study, the potential properties of stingless bee honey, *G. thoracica* and *H. itama* in wound healing, was evaluated in alloxan-induced diabetic rats through the progress of wound contraction analysis and histological evaluation.

From the macroscopic images (see ***Figure 1***) of wounds across all controls and treated groups, wound contraction consistently increased, indicating the natural progression of wound healing. Differences were observed at particular time points, during the early and proliferative phases, suggesting that stingless bee honey influences the rate of healing rather than the final outcome.

Magnification	**200 x**	**400 x**
Normal Control Group		
Diabetic Control Group		
Diabetic + *H. itama* treated group		

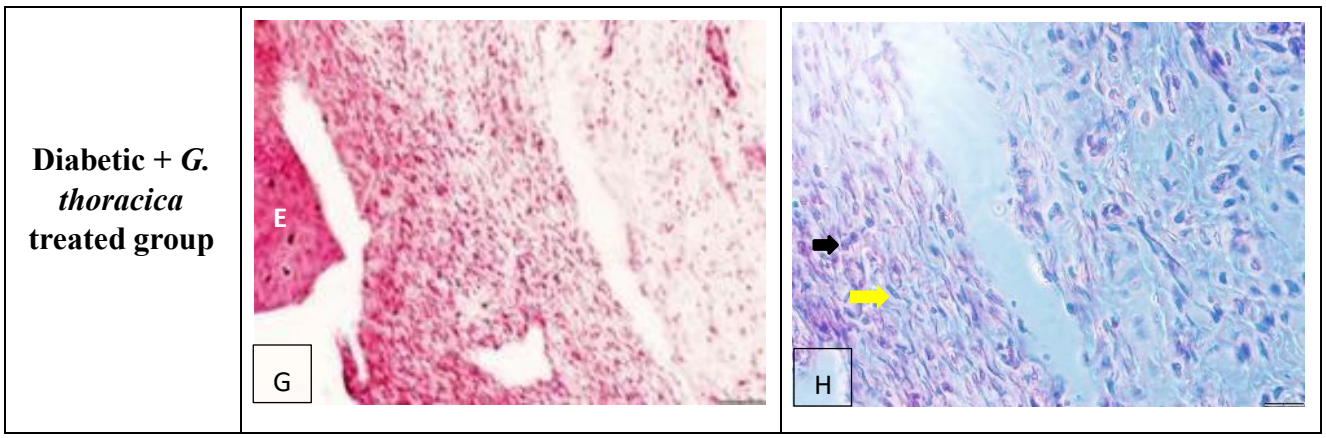


Figure 2. Histological analysis of wound tissue at Day 6 post-wounding, stained with H&E under 200× and 400× magnification. The morphology of histology on Day 6 post-wounding section. The key histological features evaluated include the infiltration of inflammatory cells, the presence of fibroblasts, collagen deposition, formation of new blood vessels, and re-epithelialization. Cellular structures are indicated as follows: fibroblasts (yellow arrow), inflammatory cells (black arrow), epidermal layer (E), blood vessels (V), and early collagen deposition (C).

A significant difference was observed on Day 3 when diabetic and honey-treated groups showed higher wound contraction than the non-diabetic group. Even though this is unexpected, early wound contraction can be influenced by edema, exudate formation, and temporary tissue contraction instead of the regeneration of real tissue.[33-35] Thus, early contraction does not necessarily indicate improved healing. The observed contraction in the honey-treated groups could be associated with early modulation of the wound environment. This can be attributed to the components present in honey which are capable of suppressing oxidative stress and inflammation.

On Day 7, the diabetic control group showed the lowest contraction value. At this stage, all wounds are undergoing the proliferative phase, where there is fibroblast proliferation, angiogenesis, collagen deposition and granulation tissue formation.[5] The relatively high SD observed in the non-diabetic group indicates biological variation, which may diminish statistical power and conceal possible differences.[36] On Day 10, wound contraction showed clearer differences between the groups, with the diabetic control group showing significantly lower contraction compared to other groups. This period is important because active tissue repair and collagen remodelling are usually taking place. The lower wound contraction observed in the diabetic control group may be due to impaired fibroblast function, reduced collagen production, and prolonged inflammation, which are common features of diabetic wounds.[32,34]

Together, these properties may contribute to a wound environment that supports healing. By the 14th day, the groups did not show significance and all the wounds were close to being completely healed. This indicates that stingless bee honey enhances the consistency and rate of healing even when the end result is the same. These results align with the previous studies that honey does not change the ultimate healing of wounds but speeds up the wound healing.[21]

For the histological evaluation, the non-diabetic control group showed features consistent with normal wound healing, including granulation tissue formation, fibroblast presence, early collagen deposition, and visible vascular structures (see ***Figures 2A*** and ***2B***). In contrast, the diabetic control group showed less organised tissue structure and fewer visible cells, which may reflect delayed healing under diabetic conditions (see ***Figures 2C*** and ***2D***). The scab-like appearance observed in the diabetic control group may also indicate incomplete re-epithelialisation and slower wound repair, which are commonly associated with prolonged

inflammation and impaired keratinocyte migration in diabetic wounds.[2] The honey-treated groups showed some histological features that appeared improved compared with the diabetic control group. In the *H. itama*-treated group, increased cell density and lumen-like structures were observed, suggesting possible early vascular formation and tissue repair activity (see ***Figure 2E***). The presence of fibroblast-like cells may indicate ongoing fibroblast migration and proliferation, which are important for extracellular matrix formation and wound contraction.[5] The *G. thoracica*-treated group also showed relatively better tissue organisation and lower inflammatory cell infiltration compared with the diabetic control group (see ***Figures 2G*** and ***2H***). Collagen deposition was also observed in the honey-treated groups, suggesting possible matrix formation during the proliferative phase. However, due to variations in the tissue orientation and section quality, the histology was used as a qualitative supporting observation only. Overall, the histological observations were generally consistent with the wound contraction data, where honey-treated groups showed improvement compared with the diabetic untreated group.

Table 1. Wound contraction on Days 0, 3, 7, 10 and 14.
Different superscript letters within the same column indicate significant differences between groups ($p < 0.05$). All values were expressed as mean and standard error mean (S.E.M). The starting experiment had n=6 animals per group. One animal was sacrificed for histological analysis at Day 6. There were n=5 animals remaining on the final day of the experiment at Day 14.

Animal Groups	**Wound contraction (%)**				
	Day 0	**Day 3**	**Day 7**	**Day 10**	**Day 14**
Non-diabetic control	0 ± 0	14.9 ± 16.34 ^{ab}	64.4 ± 9.43 ^{a}	94.7 ± 2.29 ^{a}	99.4 ± 0.94 ^{a}
Diabetic control	0 ± 0	19.1 ± 19.68 ^{a}	53.5 ± 11.27 ^{a}	82.6 ± 5.03 ^{b}	97.7 ± 2.28 ^{a}
G. thoracica	0 ± 0	23.9 ± 4.06 ^{b}	72.9 ± 6.7 ^{b}	92.3 ± 2.09 ^{a}	99.6 ± 0.55 ^{a}
H. itama	0 ± 0	34.3 ± 9.75 ^{ab}	68.5 ± 6.65 ^{a}	90.9 ± 3.34 ^{a}	99.7 ± 0.53 ^{a}

Previous studies using stingless bee honey from *Trigona* spp. have also supported its potential role in wound management. Ng et al. reported that *Trigona* honey inhibited rifampicin-resistant *Staphylococcus aureus* and helped maintain the sensitivity of *S. aureus* towards rifampicin.[37] The study also showed that the combination of *Trigona* honey and ampicillin produced stronger activity when compared to honey or ampicillin alone, suggesting a possible synergistic effect against *S. aureus*, including antibiotic-resistant strains. This supports the potential role of stingless bee honey in wound management, particularly by helping to reduce bacterial infections. This is relevant as *S. aureus* is one of the common bacteria associated with wound infection and pus formation.[38] In another study, *Trigona* honey was incorporated into a hydrogel formulation to improve its application as a wound dressing where the honey-incorporated hydrogel significantly accelerated wound closure in rabbits compared to the untreated group.[39] The

histological findings also suggest that the wound in the honey hydrogel treatment group had progressed into the remodelling phase compared with the control group that was still in the proliferation phase. Together, these findings suggest that stingless be honey from *Trigona* spp. may support wound healing through antimicrobial effects and tissue repair.

5. Conclusion

This study reports on the wound healing benefits of *H. itama* and *G. thoracica* honey in alloxan induced diabetic animal models. The results showed that topical application of stingless bee honey may support wound contraction with *G. thoracica* honey showing better wound closure. The overall trends suggest that both *H. itama* and *G. thoracica* have potential as natural agents in wound management. Further studies involving phytochemical screening, biological activities are needed to correlate the wound healing effects with their activities.

Conflict of interest

The authors declare no conflict of interest.

Ethics statement

All animal handling procedures in this study have been approved by the University Research Ethics Committee Ref: UBD/OAVCRIS/UREC/Sept2025-01(AREC).

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Appendix: Supplementary Data

Supplementary Table 1. Individual wound contraction area (cm²) and wound contraction percentage (%) of Non-diabetic control group throughout 14 days.

Rats	Days									
	0		3		7		10		14	
	Area	%	Area	%	Area	%	Area	%	Area	%
1	2.65	0	1.976	19.8946	0.786	68.136	0.300	87.838	0.00	100.00
2	2.758	0	1.519	38.421	1.002	59.380	0.195	92.095	0.00	100.00
3	2.073	0	1.443	41.50198	0.620	74.866	0.123	95.014	0.00	100.00
4	2.386	0	1.548	37.24536	0.703	71.501	0.284	88.487	0.03	98.95
AVG	2.467	0.000	1.622	34.266	0.778	68.471	0.226	90.859	0.008	99.738
SD	0.305	0.000	0.240	9.747	0.164	6.654	0.082	3.344	0.015	0.525

Supplementary Table 2. Individual wound contraction area (cm²) and wound contraction percentage (%) of Diabetic control group throughout 14 days.

Rats	Days									
	0		3		7		10		14	
	Area	%	Area	%	Area	%	Area	%	Area	%
1	1.878	0	1.791	19.3606	0.822	62.9896	0.129	94.1918	0	100.0000
2	2.286	0	2.041	-3.0617	0.487	78.0729	0.177	92.0306	0.009	99.5948
3	2.634	0	2.289	35.2094	0.896	59.6578	0.111	97.5687	0.044	98.0189
4	2.086	0	1.439	8.1045	0.956	56.9563	0.054	95.0023	0	100.0000
AVG	2.221	0	1.890	14.903	0.790	64.419	0.118	94.698	0.013	99.403
SD	0.322	0	0.363	16.342	0.209	9.431	0.051	2.288	0.021	0.943

Supplementary Table 3. Individual wound contraction area (cm²) and wound contraction percentage (%) of Diabetic group treated with *Heterotrigona itama* honey throughout 14 days.

Rats	**Days**									
	0		**3**		**7**		**10**		**14**	
	Area	**%**	**Area**	**%**	**Area**	**%**	**Area**	**%**	**Area**	**%**
1	2.356	0	1.723	35.171	1.607	39.535	0.655	86.643	0.045	98.307
2	2.572	0	1.846	30.543	1.343	49.469	0.355	83.445	0.045	94.507
3	2.627	0	2.139	19.518	0.944	64.481	0.44	85.138	0.146	99.849
4	3.076	0	2.889	-8.701	1.049	60.531	0.395	75.355	0.004	98.307
AVG	2.658	0.000	2.149	19.133	1.236	53.504	0.461	82.645	0.060	97.743
SD	0.302	0.000	0.523	19.683	0.300	11.274	0.134	5.033	0.061	2.276