A Randomized Control Trial Comparing the Effects of Manuka Honey and Tualang Honey on Wound Granulation of Post Debridement Diabetic Foot Wounds

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Summary

The concern of a surgeon dealing with the management of diabetic ulcers is to get the wound debrided and dressed until it granulates. After this stage a proper tissue cover with skin graft is among the options available to encourage the wounds to heal. A resurgence of interest and an increasing number of case reports on the use of honey on diabetic foot ulcers, reflect a growing awareness and a need for cost-effective therapies. Given honey’s great potential as an alternative in wound dressing, this double-blinded randomized controlled study was designed to investigate the wound healing property and the granulation tissue promoting effect of honey, comparing the local Malaysian tualang honey with the well-established manuka honey in the management of patients with diabetic foot wounds. Thirty-four patients with Wagner stage II or III diabetic foot ulcers were enrolled in the study, randomized into 2 groups of seventeen patients, treated with either manuka honey or tualang honey dressing on a daily basis post surgical debridement. Wound healing was assessed by measuring the granulation surface area utilizing a tracing technique. The primary outcome measure which was area of coverage with new granulation tissue was checked in each group after seven days. Other variables which might affect wound healing that were considered as confounders were recorded and analysed as well as the primary outcome. There was no significant difference (p=0.687) between manuka honey and tualang honey group in terms of mean percentage of granulation tissue surface area after one week of dressing in diabetic foot ulcers (manuka group 60.7%, tualang group 57.0%). All variables in both groups which were age, wound size, HbA1c, haemoglobin level, serum albumin level, absolute lymphocyte count and ankle-brachial systolic pressure index (ABSI), were comparable and were found not to be statistically significant to influence the primary outcome. Tualang honey induced granulation and exhibited beneficial action in promoting wound healing which was comparable to the more established manuka honey. The result suggests that tualang honey could be used as an alternative therapeutic agent for diabetic foot wounds with similar beneficial effects as those expected for manuka honey.

Keywords: Honey dressing, wound dressing, topical Honey, granulation tissue

Introduction

The surgical aim when dealing with diabetic foot infections is to eradicate the infection of the foot as soon as possible in order to salvage the foot. Most often this is achieved by combinations of antimicrobials given systemically in the acute phase, achieving good glucose control and surgical debridement. Surgical debridement is an important and effective procedure to provide a clean wound bed for the healing process to take place. It is only after a thorough debridement that application of topical wound healing agents (such as alginates or plain dressings) are carried out to promote wound closure (Steed et al., 1996). Most often split thickness skin graft will be the solution for the coverage of these wounds, however to achieve best results the wound must be covered with healthy granulation tissue otherwise the grafting might not succeed. In order to hasten this process of granulation various wound dressings are...
available commercially, of which honey based products are included. For 4000 years, the therapeutic properties of honey have been utilized for wound healing (Efem, 1988). It was first documented as a wound treatment by the Egyptians in 2000BC (Gelbart, 1999). More recently, honey has become a topic of clinical and scientific research in wound care. There is a large body of evidence to support the use of honey as a wound dressing for a wide range of types of wounds. Positive findings on honey in wound care have been reported from 17 randomized controlled trials involving a total of 1965 participants, and 5 clinical trials of other forms involving 97 participants treated with honey. The effectiveness of honey in assisting wound healing has also been demonstrated in 16 trials on a total of 533 wounds on experimental animals (Molan, 2006).

Honey has been shown to be effective in the treatment of chronic leg ulcers where the prolonged inflammatory stage of healing was resolved by the use of honey dressing (Kingsley, 2001). Molan stated that honey has a direct anti-inflammatory effect resulting in the removal of bacteria which cause the inflammation (Molan, 2006). This anti-inflammatory activity reduces oedema and exudate and prevents or minimizes hypertrophic scarring (Molan, 2001). Honey is also known to cause rapid cell stimulation (Dunford and Hanano, 2004), which helps wound healing. Honey also stimulates the growth of granulation tissue and epithelial tissue so that healing is hastened (Molan, 2006). Honey treatment significantly increased cell proliferation in the newly formed granulation tissues as reflected by DNA levels (Suguna, 1993). DNA levels which reflect fibroplasia showed maximum levels on the 8th day post injury (Aliady et al., 2000). Honey contains sugars, amino acids, minerals and vitamins, which were shown to enhance cell proliferation and hydroxyproline synthesis in the newly formed granulation tissues (Niinikoski et al., 1977). It also contains low levels of hydrogen peroxide which was found to stimulate fibroblast proliferation and angiogenesis (Chung, 1998). It is acknowledged however that the level of peroxide in honey varies between various types of honey.

The literatures indicates that honey contains components from the specific plants used by the bees in their production, and it is speculated that some of these components might further add to the antibacterial and wound-healing effects of certain honeys. The process of pasteurization, used to sterilize commercial honeys, destroys the enzyme involved in the production of hydrogen peroxide, rendering these honeys less antibacterial. Raw honeys maintain their enzymes, and honeys produced for therapeutic use are sterilized through an irradiation process that does not damage their constituents. There are currently several therapeutic honeys being marketed; two examples which can be cited are Medihoney of Australia and Active Manuka Honey of New Zealand. Both are derived from bees using the flowers of tea trees (Leptospermum spp.) as their source (Eddy et al., 2008). Manuka honey or jellybush honey derived from particular floral sources in Australia and New Zealand (Leptospermum spp) has been proved to have enhanced antibacterial activity (Molan, 2002), be effective in debriding necrotic tissue, deodorise malodorous wounds and encourage granulation and epithelialisation (Dunford and Hanano, 2004; White et al., 2006).

Manuka honey has been found to contain a naturally occurring phytochemical antibacterial factor, only found in Manuka Honey. It has been given a name: Unique Manuka Factor (UMF), also known as AMF, followed by a number indicating the strength of this Unique Factor property. The higher the factor has showed the stronger the antibacterial strength. The 'UMF' number is being the equivalent concentration of phenol with the same antibacterial activity against Staphylococcus aureus (i.e. UMF 15 = 15% w/v phenol). The New Zealand manuka (Leptospermum scoparium) honey is at present being promoted and sold as a medical product for wound dressing with substantial research to back the manufacturers' claims. Australian Leptospermum honey or Medihoney is a listed product with the Therapeutic Goods Administration in Australia and has a standardised level of this antibacterial component.

The Malaysian tualang honey has been discovered to be consistent with its content and has potential to be promoted as "medical honey" (Ainul Hafiza et al., 2005). Tualang honey gets its name from the tualang tree, Koompassia excelsa, or commonly called "Honey Bee Tree". The tualang tree is a majestic emergent tree of the Southeast Asia rainforests (it can grow as tall as 250 feet or 30 stories in height). These trees can be found growing in the lowland forests of southern Thailand, peninsular Malaysia, northeastern Sumatra, Borneo, and the Palawan islands of the Philippines. Tualang trees only branch over the canopy (until almost 100 feet up), which makes them attractive to the giant Asian rock bees Apis dorsata (the world's largest honey bees), to hang their huge disk shaped honeycombs from the horizontal branches. The combs can be 6 feet across and can contain as many as 30,000 bees. One tualang tree can contain more than 100 nests. Tualang honey is used commonly as a medicinal product and as food in Malaysia. Little scientific information about its properties has been published to date.

Since the cost of imported manuka honey to be used in patients with diabetic wound was considered, the effects of a cheaper alternative was sought. Hence in this study the effects of tualang honey was comparable to the established marketed manuka honey. The supply of tualang honey was obtained from a memorandum of understanding to undertake research on tualang honey signed between the Federal Agricultural Marketing Authority (FAMA) and Universiti Sains Malaysia (USM) in year of 2007. This provided an opportunity to exploit the honey made by wild honey bees (Apis dorsata) and to study the effectiveness of this local honey in clinical practice. FAMA had donated 230 kg of the honey to USM for research beyond the 21 uses that USM has already conducted for
treating cuts, burn wounds (Rodzaian et al., 2011; Imran et al., 2011), cancer, bone marrow (Tavafzadeh et al., 2011) and low sperm count (Mohamed et al., 2010).

**Study objectives**

The aim of the study was to study the effect of honey on granulation tissue promotion in diabetic foot ulcers. The specific objectives were as listed:

a) To measure the granulation tissue surface area of diabetic foot ulcers treated with commercially prepared “medical grade” manuka honey and local tualang honey.

b) To compare the improvement in surface area of granulation tissue granulation tissue on diabetic foot ulcers treated with manuka honey and tualang honey.

**Materials and methods**

The study was designed as a prospective randomized controlled double blinded study. The study was undertaken on patients with diabetic foot ulcers who were admitted to wards of Hospital Universiti Sains Malaysia (HUSM), a teaching hospital for the school of medical sciences of Universiti Sains Malaysia. The study period was from August 2008 to September 2009. Ethical approval was obtained from the Human research ethical committee of the medical school of the university. A study grant was obtained from the university research funding provided by the Malaysian Ministry of Higher Education under the Research Universities Programme. A written informed consent was obtained from each patient after explaining with information sheet (written in both Malay and English) about the research. The supply of manuka honey was obtained from Australia bought by retail by the principal investigator and marketed by Comvita® and sourced from the nectar of New Zealand’s native manuka flower.

The patients selected had to follow these inclusion criteria: they had to have type 2 diabetes mellitus and the wound followed the diabetic foot ulcer grading of Wagner classification grade 2 or 3 (Wagner, 1987). The patients with the following were excluded or dropped from the selection or analysis:

a) Patients with allergy to honey.

b) Patients with end-stage renal failure (ESRF).

c) Patients with serious medical illness.

d) Patients on chronic corticosteroids treatment (defined as usage of more than 2 weeks duration).

The sample size per group was calculated by comparing two means formula. The reference study for the calculation was by Mphande et al. (2007). By calculation and addition of 20 percent for dropouts, the minimum patient number required was 24 patients.

The participants were randomly assigned into two groups, namely tualang honey or manuka honey group. Block randomization method was used as this trial with two honey groups involving thirty participants. A block size of four was chosen with balanced combinations with two tualang and two manuka subjects. Blocks were randomly chosen to determine the assignment of at least 24 participants. The selection was done by the principal investigator or the research assistant. The persons who did the dressing and measurement of wound size, as well as the patient, was blinded i.e. they were not aware of the type of honey being used for the dressing. All the wound measurements were performed by a single person who was one of the co-investigators.

The measurement of the wound size and area of granulation was performed before the honey dressing started. Tracings were

**Fig.1.** The diabetic foot ulcer of a patient during wound measurement at one week and its tracing on a plastic foot wrapping placed on 1 mm squared grid for measurement.
made by placing a clean, transparent, flexible plastic sheet used for food packaging (Saran Wrap ®). The plastic film was wrapped to conform to the wound and the wound perimeter (the border between the wound floor and the peripheral epithelium) traced on the film marking with an indelible fine tipped marker. The counting procedure entailed tracing the outline of the tracing on metric graph paper (grid of smallest square of 1 mm²) and counting the number of square millimeters within the tracing. The numbers of square that appeared completely inside the tracing were counted (Fig. 1).

Wound dressing was performed by qualified staff nurses who had been well trained for the standard dressing methods. The wound was cleaned with normal saline before any dressings were applied. It was then dressed with a layer of the selected honey soaked in gauze, before a few other pieces of gauze were placed on top of that and a bandage was then applied. The dressing was performed on a daily basis for 7 days. Day seven was taken as the end point of the wound management based on the fact that the maximum granulation formed will normally be during this period. Beyond seven days the element of wound contraction and collagen formation will confound any benefits of topical agents (Fig. 2).

Participants were encouraged to stay on the ward to have the dressings done by staff nurses. However they were advised to come back for dressing everyday if they were discharged home. Participants who did not turn up for daily dressing were considered non-compliant and counted as drop-outs. Each wound was assessed daily to look for the appearance of granulation. Any deterioration or evidence of active infection assessed by wound swab culture was considered an adverse event necessitating discontinuation of the study. On day 7 the measurement procedure was repeated.

Other patient data was collected during the study recruitment and was deemed necessary for assessment of confounders and to avoid study bias. The data included were demographic details, diabetes complications, associated diseases and duration of ulcers. A neurological and vascular assessment of the foot was carried out during a physical examination. Neurological screening consisted of a sensory examination assessing the crude touch and monofilament testing. Vascular status was assessed by palpation of the dorsalis pedis and posterior tibial pulses, and was reconfirmed by hand held Doppler when the pulses were difficult to palpate. Ankle-brachial systolic pressure index (ABSI) was also measured. Laboratory biochemical testing included were current glycosylated haemoglobin level (HbA₁C) and the capillary blood sugar level on admission and other parameters for the assessment of wound healing potentials. These included the patient haemoglobin, absolute lymphocyte count and serum albumin level. Swabs taken from the diabetic foot ulcer were sent for bacterial culture and sensitivity testing. This was performed before the first dressing and after 7 days of dressing with honey. Intravenous antibiotics that was started before the surgical debridement, was continued till such time that the culture and sensitivity result of the swab was known, and the antibiotics regime was tailored accordingly to the organism(s) discovered. Antibiotics were discontinued only with confirmation of no growth via culture.

Statistical analysis utilizing univariate analysis of variance to compare the granulation tissue surface area in both groups was performed using the independent t-test. Analysis of co-variants...
(ANCOVA) was used to compare the effect of the two types of honey on the improvement percentage of granulation tissue surface area after controlling on variables and also the baseline sizes. The statistical software used for data entry and analysis was SPSS Version 12.0.1 for Windows licensed to Universiti Sains Malaysia (906085). A value of p<0.05 was taken as statistically significant.

Results

A total of 34 patients (more than the number required) were enrolled in the study, 11 were males and 23 were females. The youngest patient was 34 years old and the eldest was 75 years old. Thirteen patients (38.2%) were in the age group 50-59 years. There were 17 patients in the tualang honey treated group (TG) with the mean age of 53.9 years. The other 17 patients were enrolled into the group treated with manuka honey dressing (MG). In this group, the mean age was 52.5 years, ranged from 41 to 67 years. In the histogram distribution, both were found to be normal. The sex distribution in TG group was 12 females (70.6%) and 5 males (29.4%), while 6 males (35.3%) and 11 females (64.7%) in MG group. The diabetic foot ulcers were distributed almost equally in both groups according to the Wagner’s classification. TG group had nine grades II and eight grade III diabetic foot ulcers. Meanwhile the MG group made up of ten (grade II) and seven (grade III) ulcers respectively.

The region most involved in both the groups was noted to be at the forefoot, following surgical debridement or ray amputation of the digit, making up 47.1% of total number of patients. The figure was followed by the area involving hind foot, which comprised of 6 patients (17.7%). Midfoot involvement was found in 5 patients (14.7%). The large ulcers involving either forefoot to midfoot or midfoot to hind foot were not uncommon in this series, with 17.7% and 2.9% respectively.

Table 1 shows the healing parameters of the selected study subjects; they were not significantly different to influence the study outcome as shown in table 2.

The mean wound size before the honey dressing i.e. day 0 was 3007.7 mm² (TG group) and 3855.7 mm² (MG group). After dressing for 7 days the mean percentage of improvement in terms of granulation surface area was 59.3% in wounds dressing with tualang honey and 58.0% in wounds dressed with manuka honey as shown in table 2, they were not statistically significant (Table 3).

To test a multivariate model involving multiple linear variables, the ANCOVA (analysis of co-variants) was performed. This was to test the significance of co-variants and to quantify the influence of these confounders on the end-point in question, which is the improvement of granulation tissue at day 7 of honey dressing being the model. The previously mentioned categorical variables were wound size on day 1, granulation area on day 1, HbA1c, ankle-brachial systolic pressure index, Haemoglobin level, absolute lymphocytes count, and serum Albumin level. The results are as presented in Table 4.

Table 1. Comparison of healing parameters in both groups (using independent t-test).

<table>
<thead>
<tr>
<th>Healing parameter</th>
<th>Tualang honey (n=17)</th>
<th>Manuka honey (n=17)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c (%)</td>
<td>11.4 ± 2.2</td>
<td>10.3 ± 2.1</td>
<td>0.122</td>
</tr>
<tr>
<td>Ankle-brachial systolic pressure index</td>
<td>1.04 ± 0.17</td>
<td>0.96 ± 0.17</td>
<td>0.160</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>32.2 ± 4.5</td>
<td>31.5 ± 4.4</td>
<td>0.617</td>
</tr>
<tr>
<td>Lymphocytes (/mm³)</td>
<td>2605.9 ± 525.0</td>
<td>2471.7 ± 578.4</td>
<td>0.484</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>11.2 ± 1.1</td>
<td>11.2 ± 1.5</td>
<td>0.990</td>
</tr>
</tbody>
</table>

Table 2. Univariate analysis (Independent t-test) performed to ascertain comparability of cohorts.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Significance (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound size on day 1 (mm²)</td>
<td>0.176</td>
</tr>
<tr>
<td>Granulation area on day 1 (mm²)</td>
<td>0.779</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>0.687</td>
</tr>
<tr>
<td>Ankle-brachial systolic pressure index</td>
<td>0.160</td>
</tr>
<tr>
<td>Haemoglobin level (g/dL)</td>
<td>0.990</td>
</tr>
<tr>
<td>Serum Albumin level (g/L)</td>
<td>0.617</td>
</tr>
<tr>
<td>Absolute Lymphocytes count (/mm3)</td>
<td>0.484</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>0.122</td>
</tr>
</tbody>
</table>

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**Table 3.** Comparison of percentage of improved granulation surface area in both the cohorts after 7 days of dressing (Independent t-test).

<table>
<thead>
<tr>
<th></th>
<th>Tualang honey (n=17)</th>
<th>Manuka honey (n=17)</th>
<th>p value</th>
<th>Mean Difference</th>
<th>95% Confidence Interval of the Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean percentage of</td>
<td>59.3 ± 26.0</td>
<td>58.0 ± 25.8</td>
<td>0.884</td>
<td>1.30</td>
<td>Lower -16.8</td>
</tr>
<tr>
<td>improved granulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Upper 19. (Equal variances assumed)</td>
</tr>
<tr>
<td>surface area (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Based on adjusted means. Adjustment for multiple comparisons: Bonferroni.

**Table 4.** The analysis of mean percentage of improved granulation surface area (%) after all the previously mentioned categorical variables were considered in the ANCOVA.

<table>
<thead>
<tr>
<th></th>
<th>Tualang honey (n=17)</th>
<th>Manuka honey (n=17)</th>
<th>p value</th>
<th>Mean Difference</th>
<th>95% Confidence Interval of the Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean percentage of</td>
<td>57.0 ± 5.5</td>
<td>60.7 ± 5.5</td>
<td>0.678</td>
<td>-3.4</td>
<td>Lower -20.2</td>
</tr>
<tr>
<td>improved granulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Upper 13.4</td>
</tr>
<tr>
<td>surface area (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Based on adjusted means. Adjustment for multiple comparisons: Bonferroni.

**Discussion**

This study primarily aimed at investigating and comparing the effectiveness of tualang and manuka honey in promoting granulation tissue in diabetic foot ulcers. Tualang honey however has never been widely demonstrated in the medical literature as a wound dressing material, which is in contrast to manuka honey, which has been graded as a medical honey and is well established in the literature. Tualang honey is readily available in Malaysia, but its quality and floral origin have yet to be determined and standardized (Tan et al., 2009). In contrast, manuka honey of New Zealand has been widely researched and its antibacterial and wound healing potential is renowned worldwide. Therefore, manuka honey was chosen as a comparison for this study of the diabetic foot ulcer healing potential of tualang honey.

The ability of topically applied honey to stimulate tissue growth in wounds was checked by measurement granulation tissue surface area after 7 days of honey dressing. It was observed that no significant difference (p=0.678) occurred between these two groups in the improvement of the percentage of granulation tissue surface area, even though the result of manuka group was 60.7%, compared to tualang group, 57.0%. These data suggested that the beneficial action of tualang honey in wound healing was comparable to manuka honey. Other varieties of honeys might show the same benefit, however that was beyond the scope of this study.

Generally, the patient acceptance of honey was positive as local raw honey has been commonly used as an alternative therapy in rural community. Some might feel uncomfortable applying a sticky food substance on to their foot ulcers and some experienced mild pricking sensation on the initial dressing procedures. An overall review of these 34 patients using honey for diabetic foot ulcers had showed positive outcomes with encouraging patient acceptance. The authors were also not aware of any local or systemic atopic reaction related to the use of honey in any of the patients. This suggested that honey was a safe and satisfying healing agent when used topically on wounds.

The strength of this study was its double-blinded, randomized control nature. The study could however, have been improved if it had a placebo controlled arm. However it was near impossible to conduct a double-blinded trial with a placebo control against honey as a wound dressing, because of the difficulty of keeping the placebo or control material obscured from the patients and the person involved in performing the dressing procedure. Even if honey was well kept in a bottle covered to prevent direct vision, its colour was readily differentiated once it was applied to the ulcers. In addition, its aroma was immediately recognised, causing even a single blind randomized trial difficult to conduct (Molan, 2006).

A clean and healthy wound bed provides basis in wound healing. It promotes the formation of granulation tissue and hastens epithelialisation. The authors chose to have patients with diabetic foot ulcers fresh post surgical debridement. The radicalism and thoroughness of the debridement could be “surgeon-dependent”. This might be hampered by fear of devascularising deeper structures and was a quality difficult to render uniformity. Thus, to attain the same level of reduction of bio-burden in all these wounds was virtually impossible, seeing the heterogeneity of the wounds involved and the number of different surgeons who were responsible for their debridement.

Tualang honey showed its granulation promoting effect was comparable to manuka honey in this study. The concern of the
authors is the reproducibility of the composition and properties of tualang honey. It is necessary to be aware that honey is a natural product, and that those characteristics associated with wound healing may be affected by species of bee, geographical location and botanical origin, as well as processing and storage conditions (Moore et al., 2001). The difference between tualang honey and the others is that the honeybees are wild and feed on different types of flowers. This is in contrast to farmed bees, which are fed sugar on top of flowers of particular species. Further biochemical and biophysical studies may help elucidate which specific tualang honey is the most consistent with its contents and activities.

Diabetic foot ulcers are undoubtedly very complicated, and honey would only be part of the solution. This was clearly shown in the mean improvement of granulation in both cohorts merely past 55%. Successful care requires optimizing all the wound-healing parameters, off-loading on the ulcer, reducing microbial burden and the thorough surgical debridement of initial infected ulcers. Interestingly, Aljady et al., (2000) demonstrated combined honey treatment (topical and oral) offers a distinct advantage to wound healing. This was however an animal study using another Malaysian honey, "Gelam" honey. Further study in future with tualang honey may clarify more issues on this adjuvant therapy.

Tualang honey can be considered as an alternative to imported manuka honey with equal ability to aid healing. To determine if it is better or equal to modern wound dressings needs to be verified with other studies.

Acknowledgements

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