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ORIGINAL RESEARCH ARTICLE

The Efficacy of Tualang Honey in Comparison to Silver in Dressing Wounds in Rats

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Summary

Tualang honey is obtained from large honeycombs produced by Asian bees (*Apis dorsata*) in gigantic Tualang trees. It has been used traditionally by local communities to treat wounds. However, unlike manuka honey its medicinal uses are not well researched. An open, prospective study into the efficacy of wound healing in full thickness wounds in rats, was designed to compare two honey impregnated dressings with silver-impregnated hydrofibre dressings. A full-thickness wound was created on the dorsum of Sprague-Dawley rats (n=45). Tualang honey impregnated paraffin tulle (P-honey) and tualang honey impregnated hydrofibre dressings (H-honey) were compared with silver-containing hydrofibre dressing (positive control; H-Ag). The wounds were inspected on days 4, 7, 14, 21 and 28. The dressings and wounds were assessed for adherence, ease of removal, fluid accumulation, dryness of skin and exudates, rate of epithelization, healing and wound contraction. Three rats treated with each dressing were sacrificed on the days that wounds were inspected. The wounds and scars were histologically analysed for inflammatory parameters. Tualang honey impregnated dressings were comparable to the commercially available silver impregnated hydrofibre dressing in terms of adherence, ease of removal, fluid accumulation, dryness of surrounding skin and exudates; $p > 0.05$ for non-parametric Kruskal-Wallis tests and post hoc corrections with a Mann-Whitney test. The rates of wound healing, wound contracture and subsequent histological analysis of inflammatory reaction by each dressing were also comparable. Tualang honey impregnated dressings were as effective as silver impregnated hydrofibre dressings in terms of dressing properties, promotion of wound healing and inflammatory reaction.

Keywords: Tualang honey, silver dressing, hydrofibre dressing, full thickness wound, wound healing.

Introduction

Honey has been used as a wound treatment for over 4000 years. One of the earliest writings on honey in the Edwin Smith Surgical Papyrus was dated before 1600 BC. The healing power of honey has regained recognition by the scientific community in the last fifteen years (Molan, 2000). Since then, honey has been used for the treatment of chronic wounds, burns, methicillin-resistant *Staphylococcus aureus* (MRSA), necrotic wounds, malignant ulcers and many other ailments (Maeda *et al.*, 2008; Visavadia *et al.*, 2008; Blaser *et al.*, 2007; Shukrimi *et al.*, 2008; Simon *et al.*, 2009; Gethin and Cowman, 2005).

In a 2008 Cochrane review of topical honey treatment, Jull *et al.* (2008) found that honey might improve healing times for mild to moderate superficial and partial thickness burns compared to conventional dressings. However, honey did not significantly increase leg ulcer healing time when it was used as adjuvant therapy. Furthermore, there was insufficient evidence to guide the use of honey in clinical practice in all other areas.

Honey is thought to have many advantages in promoting wound healing. For example, honey has antimicrobial properties against MRSA infections (Maeda *et al.*, 2008; Blaser *et al.*, 2007; Norizah *et al.*, 2004), it is able to deslough or debride necrotic tissues (Visavadia *et al.*, 2008), it may act as anti-inflammatory agent, it can

stimulate healing (Tonks *et al.*, 2003) and can reduce the occurrence of malodorous wounds. The use of honey as a wound dressing can be affordable for various socio-economic groups and is cost-effective compared to many types of modern dressings (Ingle *et al.,* 2006; Moghazy *et al.*, 2010).

Asian giant honeybees, *Apis dorsata*, produce tualang honey in the rainforest of Malaysia. The honeybees nest on the gigantic Tualang tree, *Koompasia excelsa* (Crane, 1999). Tualang honey has many uses in the local community including wound treatment, beauty products, anti-ageing products and health supplements. Several studies have been conducted on this rainforest honey (Norizah *et al.*, 2004; Kannan *et al.*, 2009) with the goal of emulating the success of manuka honey in medical field (Visavadia *et al.*, 2008; Gethin and Cowman, 2005 and 2009; Jull *et al.*, 2008). So far, there is still a lack of data on the efficacy of tualang honey on wound healing.

Wound healing usually progresses in a tightly regulated manner through four overlapping phases. Healing begins immediately upon wounding with haemostasis. Haemostasis is followed by an inflammatory phase (day 0 up to 1 week) and then a proliferative (fibroplasia) phase (from days 2-3 and to 2-4 weeks). Healing ends with a remodeling phase (beginning at approximately 3 weeks after wounding) that may take more than twelve months in duration. Ideally, there should be a controlled inflammatory reaction to debride the wound of damaged tissue and contaminating microbial cells, but without an over zealous inflammatory response that may turn into chronic inflammation and subsequently prolong wound healing (Rohrich and Robinson, 1999).

Appropriate wound management protocols may aid wound healing. One desirable wound dressing property is good adherence. The dressing should have excellent contact with the wound bed to eliminate the dead space that can become a nidus for bacterial multiplication. A dressing should also be easily removable to allow for wound inspection and dressing changes with minimal discomfort to the patient. This quality will minimize trauma to the newly formed epithelium. Moisture control is also an important factor in a wound-healing environment. Therefore, a dressing that can withstand a moist environment and is able to absorb excessive fluid discharge from the wound is desirable.

Tualang honey is one of the most widely available honeys in our local setting, it is inexpensive and has been traditionally used for medicinal purposes and for the treatment of wounds. Therefore, in this study we aimed to compare the efficacy of each of the two tualang honey impregnated dressings, hydrofibre (H-honey) and paraffin tulle (P-honey), with a silver impregnated hydrofibre (H-Ag) dressing in a rat model. The H-Ag dressing was used as a positive control in this study because it is one of the best available modern dressings. It has an excellent fluid retaining property that locks the water in its gelling form. This can prevent surrounding skin maceration and facilitates the entrapment of microorganisms within the dressing itself. H-Ag dressing also conforms easily to the wound surface and has good adherence with the wound bed. It can be easily removed from the wound bed without pain and its silver component has been reported to have antimicrobial properties (Hoekstra *et al.*, 2002; Walker *et al.*, 2003).

Materials and methods

An animal model was used to study the effect of tualang honey impregnated dressing on a full-thickness wound. The sample size was calculated based on previous study by Khan and Peh, (2003) which used 3 rats per subgroup. Here rats were randomized into three groups and further divided into five subgroups with the total number of rats being forty five. Male Sprague Dawley rats that weighed between 250 to 300 g were used in this study. The rats were each housed in an individual cage with free access to water and food in the same environment to avoid baseline discrepancies. The study was approved by the local Animal Ethical Committee.

The rats were anaesthetized using intramuscular ketamine 35 mg/kg-1 and hydralazine 5 mg/kg. The dorsum of the rats was shaved and prepared with povidone-iodine antiseptic solution. A 10 by 10 mm square was marked on the centre of the dorsum of each rat when they were in a relaxed condition. A full thickness incision was made with a size 15 scalpel and the resultant wound was measured (after retraction) to verify the actual wound size at day zero.

The silver impregnated hydrofibre (H-Ag) dressing was cut to cover the wound and slightly overlap with the surrounding skin by approximately 5 mm. The hydrofibre and paraffin tulle were similarly cut and soaked in Agromas® Tualang honey until all parts of the dressing materials were fully impregnated (H-honey and P-honey respectively). Approximately 1 - 2 mL of honey was used for each dressing. After each dressing was applied to the wound, it was covered by gauze as a secondary dressing and sutured to the surrounding rat skin using Mersilk® 4/0. Finally, the dressing was wrapped by a bandage to prevent any interference or displacement of the dressing due to the rats' activities.

The wounds were inspected and changed on days 4, 7, 14, 21 and 28, until complete epithelization was achieved. Nine rats were sacrificed on each inspection day before the wound or scar samples were taken. Sacrificed rats were used for histological examination using haematoxylin and eosin staining. Specimens taken on each inspection day were examined for tissue responses (inflammatory cells, angiogenesis and collagen deposition) at the different phases of wound healing as described above. The wound and/or the resultant scar was excised with 5 mm to 10 mm surrounding normal skin, and including the underlying muscular layer. The specimen was then fixed in 10% formalin, and a histological slide was prepared using hematoxylin and eosin (H & E) staining.

Table 1. Scoring system for the evaluation of the performance of the dressing and condition of the wound.

		Scores				
Dressing evaluation						
Adherence	Non-adherent	0	1	2	3	Good adherence
Ease of removal	Difficult	0	1	2	3	Easy
Fluid accumulation	Yes	0	1	2	3	No
Wound evaluation						
Dryness of skin	Wet	0	1	2	3	Dry
Exudation	High exudate	0	1	2	3	No exudate
Odour	Bad	0	1	2	3	Good

Dressings performance and the wound condition was evaluated by a single, un-blinded observer (Table 1) (Khan and Peh, 2003).

In addition to wound area measurement, the percentage of scar contraction was calculated using the following formula (Khan and Peh, 2003):

$$\text{Scar contractive} = \frac{WA_{\text{Day }0} - SA_{\text{Day X}}}{WA_{\text{Day }0}} \times 100\%$$

Where:

X = Time in days after wound creation

WA = Wound area

SA = Scar area

The wound healing was measured by means of re-epithelization and reduction of the unclosed area. This was done by a single investigator to avoid inter-observer variability and bias. The wound area and percentage of epithelization were measured on days 4, 7, 21 and 28, just before the rats were sacrificed. The percentage of epithelization was calculated using the following equation (Khan and Peh, 2003):

$$\text{Wound epithelialization} = \frac{SA_{\text{Day X}} - WA_{\text{Day X}}}{SA_{\text{Day X}}} \times 100\%$$

Where:

X = Time in days after wound creation

SA = Scar area

WA = Unclosed wound area

Histological examination of the wounds and scars was conducted blind using coded specimens. The specimens were assessed for the presence of inflammatory cells (neutrophils, macrophages and lymphocytes), angiogenesis, fibroblasts, collagen, amount of surrounding tissue infiltration with inflammatory cells and the presence of skin appendages (such as hair follicles and sebaceous glands). The amount of these cells or structures present in the wound was scored using an arbitrary unit (Table 2).

Table 2. Scoring guide for histological examination

Assessment of cell type, blood vessels, amount of collagen, and hair follicles/sebaceous glands:
0 = cell or structures not present 1 = low number (1-10 / hpf*) 2 = moderate (11 - 20 / hpf*) 3 = cells present at a relatively high number (>20 / hpf*)

* hpf = high power field

Statistical Analysis

The results obtained from wound assessment, dressing assessment and histological examination were analysed using a non-parametric Kruskal-Wallis test. A similar test was used to analyze the degree of scar contraction and the percentage of epithelization. When statistically significance differences were obtained ($p<0.05$) post hoc analysis was performed using a Mann-Whitney test for multiple comparisons with a corrected p-value. All the statistical analyses were calculated using SPSS version 12.0.1 (Apache Software Foundation, US, 2003).

The study was conducted as an open-labelled manner where the assessment was performed by the principal investigator alone. However, in the second part of the study, an independent, blinded observer was used for histological analysis.

Results

The tualang honey impregnated hydrofibre (H-honey) dressing consistently outscored the H-Ag dressing and the tualang honey impregnated paraffin tulle (P-honey) in the dressing assessment. The mean scores of all of these dressings were between two and three, which signified moderate to good qualities. There were significant differences in dressing adherence and ease of dressing removal on day 4, 7 and 14 (Kruskal-Wallis test, $p<0.05$; Table 3).

Table 3. Assessment of the dressings for adherence, ease of removal and fluid accumulation (mean ± SD).

Dressing/criteria	Scores			
	Day 4	**Day 7**	**Day 14**	**Day 21**
Adherence				
Hydrofibre/silver	2.7 ± 0.6	2.8 ± 0.6	3.0 ± 0.0	3.0 ± 0.0
Paraffin tulle/honey	1.9 ± 0.9	2.1 ± 0.9	2.4 ± 0.9	3.0 ± 0.0
Hydrofibre/honey	2.7 ± 0.5	2.9 ± 0.3	3.0 ± 0.0	3.0 ± 0.0
Statistical significance	p=0.02 *	p=0.01 *	p=0.039 *	p>0.95
Ease of removal				
Hydrofibre/silver	2.6 ± 0.6	2.8 ± 0.6	3.0 ± 0.0	3.0 ± 0.0
Paraffin tulle/honey	2.1 ± 0.8	2.2 ± 0.9	2.6 ± 0.7	3.0 ± 0.0
Hydrofibre/honey	2.8 ± 0.4	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0
Statistical significance	p=0.02 **	p=0.006 **	p=0.039 *	p>0.95
Fluid accumulation				
Hydrofibre/silver	2.5 ± 0.6	2.7 ± 0.6	2.9 ± 0.3	2.8 ± 0.4
Paraffin tulle/honey	2.2 ± 0.6	2.3 ± 0.6	2.6 ± 0.5	2.3 ± 0.5
Hydrofibre/honey	2.6 ± 0.5	2.7 ± 0.5	2.8 ± 0.4	2.7 ± 0.5
Statistical significance	p=0.24	p=0.25	p=0.273	p=0.213
Dryness of skin				
Hydrofibre/silver	2.6 ± 0.5	2.7 ± 0.5	2.7 ± 0.5	2.5 ± 0.5
Paraffin tulle/honey	2.3 ± 0.7	2.5 ± 0.7	2.6 ± 0.5	2.5 ± 0.5
Hydrofibre/honey	2.6 ± 0.5	2.6 ± 0.5	2.8 ± 0.4	2.7 ± 0.5
Statistical significance	p=0.46	p=0.6	p=0.843	p=0.809
Exudation				
Hydrofibre/silver	2.4 ± 0.5	2.6 ± 0.5	2.7 ± 0.5	2.7 ± 0.5
Paraffin tulle/honey	2.3 ± 0.6	2.3 ± 0.6	2.6 ± 0.5	2.5 ± 0.5
Hydrofibre/honey	2.5 ± 0.5	2.6 ± 0.5	2.6 ± 0.5	2.5 ± 0.5
Statistical significance	p=0.57	p=0.539	p=0.858	p=0.809

* Post hoc correction with Mann-Whitney test – $p > 0.05$.

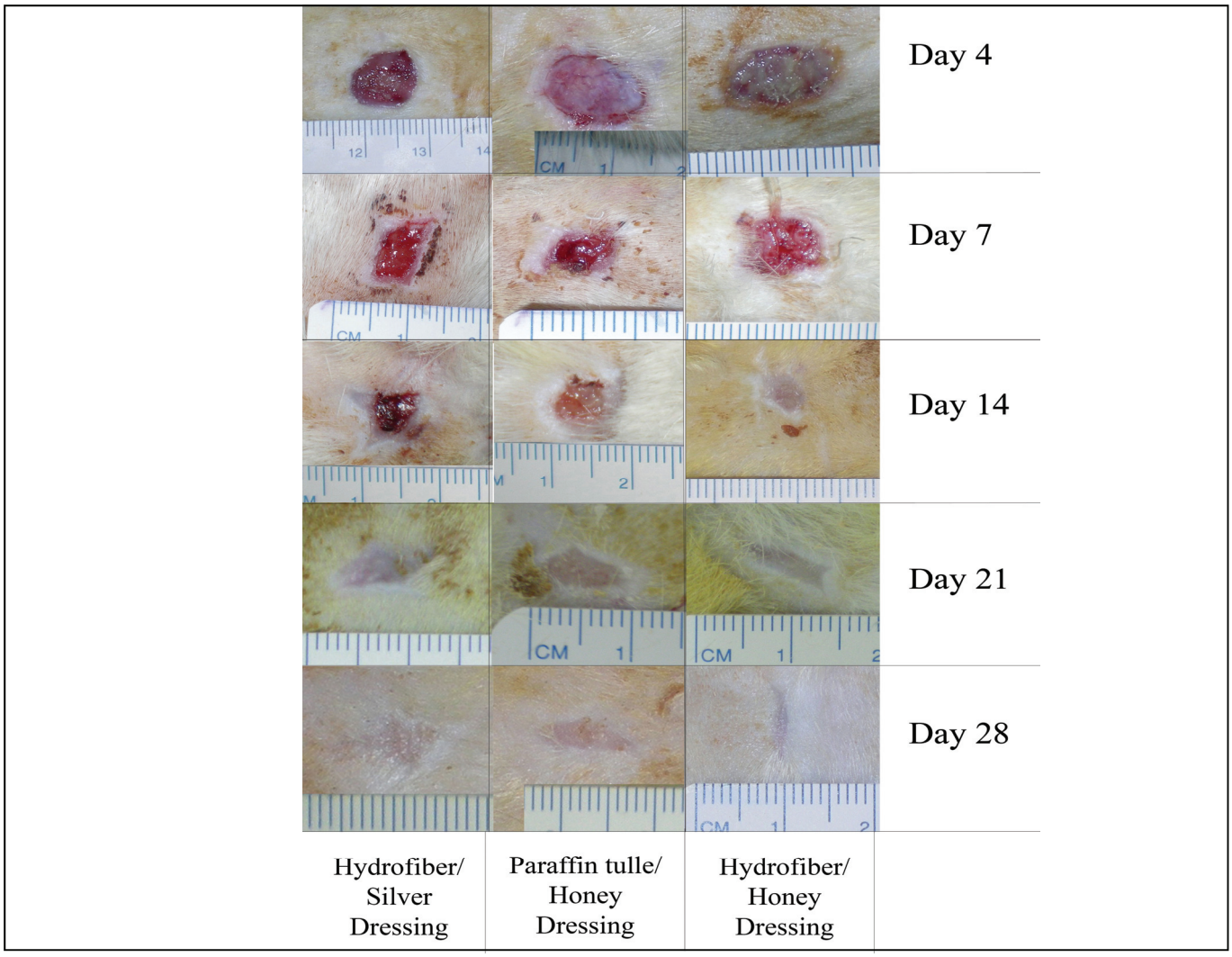

Fig. 1. Appearance of wounds and scars on days 4, 7, 14, 21 and 28 under the three dressing types.

Table 4. Measurement of wound area, percentage of epithelization and percentage of scar contracture (mean ± SD).

	Day 0	Day 4	Day 7	Day 14	Day 21	Day 28
Wound area (mm^2)						
Hydrofibre / silver	137 ± 22	78.3 ± 13.2	28.6 ± 23.8	10.0 ± 9.5	-	-
Paraffin tulle / honey	141 ± 29	104.6 ± 30.3	28.6 ± 23.8	12.0 ± 10.5	-	-
Hydrofibre / honey	140 ± 25	70.6 ± 17.0	29.0 ± 13.5	5.3 ± 6.1	-	-
Statistical significance	p=0.99	p=0.252	p>0.95	p=0.658	-	-
% of epithelization						
Hydrofibre / silver	0	36.2 ± 23.5	50.0 ± 40.0	71.7 ± 25.8	100	100
Paraffin tulle / honey	0	11.5 ± 19.9	43.9 ± 5.5	66.3 ± 29.2	100	100
Hydrofibre / honey	0	37.0 ± 9.2	55.4 ± 15.5	82.2 ± 20.3	100	100
Statistical significance	-	p=0.190	p=0.561	p=0.661	p>0.95	p>0.95
% of scar contracture						
Hydrofibre / silver	0	18.0 ± 5.4	61.2 ± 4.4	72.9 ± 2.8	84.5 ± 12.6	78.3 ± 10.1
Paraffin tulle / honey	0	31.7 ± 8.2	63.3 ± 22	68.1 ± 6.1	79.0 ± 6.8	88.1 ± 4.3
Hydrofibre / honey	0	19.8 ± 11.0	49.0 ± 8.2	74.3 ± 10.9	80.6 ± 4.9	78.4 ± 11.1
Statistical significance	-	p=0.249	p=0.301	p=0.561	p=0.670	p=0.193

Table 5 . Mean count (± SD) of inflammatory cells, fibroblasts, collagen and skin appendages in histological examination.

Day	4	7	14	21	28
Angiogenesis					
Hydrofibre / silver	2.3 ± 0.57	1.6 ± 0.57	0.33 ± 0.28	1.167 ± 0.76	0.833 ± 0.28
Paraffin tulle / honey	1.67 ± 0.57	2.16 ± 0.76	1.33 ± 0.28	2.33 ± 0.57	1 ± 0.5
Hydrofibre / honey	2.33 ± 0.57	2.6 ± 0.57	1 ± 1.00	1.33 ± 0.57	1.33 ± 0.76
P value	p = 0.3	p = 0.24	p = 0.18	p = 0.15	p = 0.59
Neutrophils					
Hydrofibre / silver	2.667 ± 0.57	1.167 ± 0.76	2.333 ± 1.15	0.5 ± 0.86	0.5 ± 0.5
Paraffin tulle / honey	1.833 ± 0.28	1.5 ± 1.32	1.167 ± 1.61	1 ± 1.73	0.333 ± 0.28
Hydrofibre / honey	2.333 ± 1.15	1.833 ± 1.25	0.833 ± 1.44	0.5 ± 0.86	1.167 ± 1.61
P value	p = 0.34	p = 0.83	p = 0.23	p = 0.95	p = 0.85
Macrophages					
Hydrofibre / silver	2.333 ± 0.57	1.5 ± 0.5	3 ± 0	1.5 ± 0	1.5 ± 0
Paraffin tulle / honey	2 ± 0	1.667 ± 0.57	2.167 ± 0.76	2.667 ± 0.57	2.5 ± 0.5
Hydrofibre / honey	2 ± 0	2.167 ± 0.28	2.333 ± 0.57	1.5 ± 0.5	2 ± 0
P value	p = 0.36	p = 0.27	p = 0.21	p = 0.06	p = 0.03*
Lymphocytes					
Hydrofibre / silver	2 ± 1.0	1.833 ± 0.28	2.333 ± 0.57	2 ± 0	2.167 ± 0.28
Paraffin tulle / honey	2.667 ± 0.57	2.333 ± 0.76	1.667 ± 0.28	2.5 ± 0.86	3 ± 0
Hydrofibre / honey	1.833 ± 0.28	2.667 ± 0.57	2.333 ± 0.76	2 ± 1.0	2.333 ± 0.57
P value	p =0.29	p = 0.26	p = 0.27	p = 0.71	p = 0.09
Fibroblasts					
Hydrofibre / silver	3 ± 0	2.5 ± 0.86	2 ± 0	2.5 ± 0.5	2.333 ± 0.57
Paraffin tulle / honey	1.8 ± 0.28	1.83 ± 1.25	2 ± 0	2.833 ± 0.28	2.333 ± 0.57
Hydrofibre / honey	2.667 ± 0.57	3 ± 0	2.333 ± 0.57	2.167 ± 0.28	2.667 ± 0.28
P value	p = 0.56	p = 0.28	p = 0.37	p = 0.16	p = 0.54
Collagen					
Hydrofibre / silver	1.333 ± 0.57	1.833 ± 0.28	2.0 ± 1.0	2.333 ± 0.57	3.00 ± 0
Paraffin tulle / honey	1.833 ± 0.28	1.167 ± 0.76	2.333 ± 0.57	1.333 ± 0.57	2.833 ± 0.28
Hydrofiber / honey	1.333 ± 0.57	1.167 ± 0.28	2.667 ± 0.57	2.0 ± 0	2.333 ± 0.57
P value	p = 0.36	p = 0.21	p = 0.56	p = 0.11	p = 0.19
Surrounding tissue infiltration by inflammatory cell					
Hydrofibre / silver	1.333 ± 0.57	1 ± 0	1 ± 0.86	1.333 ± 0.57	1 ± 0.5
Paraffin tulle / honey	0.833 ± 0.57	0.833 ± 0.28	0.5 ± 0.5	1.667 ± 0.57	1 ± 0.5
Hydrofibre / honey	1.5 ± 0.86	0.667 ± 0.28	1.167 ± 0.57	0.5 ± 0.5	0.833 ± 0.28
P value	p = 0.4	p = 0.26	p = 0.44	p = 0.71	p = 0.85
Presence of skin appendages					
Hydrofibre / silver	0	0	0	0.3333 ± 0.57	0.6667± 1.15
Paraffin tulle / honey	0	0	0	0.6667 ± 1.15	1.6667± 0.57
Hydrofibre / honey	0	0	0	0.3333 ± 0.57	0
P value	p > 0.95	p > 0.95	p > 0.95	p = 0.95	p = 0.09

* Post hoc correction with Mann-Whitney test, p > 0.05.

Subsequent post hoc analyses showed that the only statistically significant parameter was the ease of removal between the H-honey and P-honey dressings on days four ($p = 0.039$) and seven ($p = 0.018$). There was no significant difference found between the two tualang honey dressings and the control dressing.

The percentage of epithelization was best in the H-honey dressing, followed by the H-Ag dressing and the P-honey dressing. All wounds healed completely by day 21 (Fig. 1). There were no statistical differences between the dressings in term of healing time (Table 4). The percentage of wound epithelization increased steadily until wounds were fully epithelized or healed. In contrast to rate of epithelization, the P-honey dressing showed better wound contraction, especially in the early period, compared to the other two dressings. However, this was not statistically significant.

Angiogenesis was mainly observed in the first week in all groups and began to decrease as healing progressed. The honey dressings were marginally better than those in the control group but this difference was not statistically significant (Table 5).

The tualang honey impregnated dressings showed a lower neutrophil count compared to the H-Ag dressing. However all study groups showed similar presence of macrophages in the wound throughout the study. A statistically significant difference in macrophage count was found between the dressings only on day 28. However, post hoc analysis with Mann-Whitney tests and corrected p-values showed no significant difference among the groups.

The fibroblast counts were initially high in all of the wounds. They subsequently were reduced as the collagen fibres increased in the wound matrix. The amount of inflammatory cells infiltrating the surrounding tissue was low in all dressings, with a score between 0.5 and 1.6. The presence of skin appendages detected in the scar by day 21 was highest in the P-honey dressing. There were no statistical differences between the dressings.

Discussion

Honey dressings aid the wound healing process in several ways. The advantages of honey as a dressing include its antimicrobial properties (Maeda *et al.*, 2008; Blaser *et al.*, 2007; Norizah *et al.*, 2004), its high osmotic pressure that can absorb wound exudates thus keeping the surrounding area dry, its debridement ability (Visavadia *et al.*, 2008) and its anti-inflammatory property. Honey dressings also stimulate healing (Tonks *et al.*, 2003) and can reduce the occurrence of malodorous wounds.

It is believed that different types of honey do not have the same efficacy in inhibiting microorganisms or in their ability to maintain their antimicrobial activity upon dilution or removal of hydrogen peroxide. Different types of honey also have differing phytochemical content (Allen *et al.*, 1991). The differences in the antimicrobial activity of various honeys are believed to be due to their different floral origins. Some studies have been done on honeys from different flora origins to evaluate the differences in sugar content, amino acid content and other components. These compositional differences may influence the value of a specific honey for medicinal or health-promoting purposes (Pawlowska and Armstrong, 1994; Flodhazi, 1994).

A dressing must have the ability to rapidly and uniformly adhere and conform to wound bed contour to prevent air or fluid pocket formation. Good adherence can prevent peripheral migration of microorganisms into the wound, create a closed environment for the wound, promote bonding to tissue, decrease movement and shearing and reduce pain (Khan and Peh, 2003). Additionally, the dressing must be easily removed from the wound for wound inspection. This can prevent any damage to the wound surface that is undergoing the healing process. The ability of a dressing to absorb fluid exudates from the wound while retaining the moist environment will aid wound healing. The surrounding skin condition can be a predictor of how well a dressing absorbs exudates. This is important in order to prevent wound deterioration. Wound deterioration is indicated by maceration, followed by inflammation of the surrounding skin if the environment is overly wet and bathed by wound exudates.

A dressing must also be inexpensive and easily available in order for it more accessible to all socio-economic groups of patients. AgroMas® Tualang honey costs approximately $3.50 USD for every 230 g bottle of honey ($0.05 USD to prepare one dressing 2 x 2 inches). This is much more affordable than many modern dressings currently available in the market, such as hydrofibre impregnated silver dressing that costs approximately $1.50 USD per sheet (2 x 2 inches).

The silver impregnated hydrofibre dressing was used in this study as a control to match the antibacterial property possessed by the tualang honey dressing (Nasir *et al.*, 2010) even though they were clean surgically created wounds. Therefore, the study was comparable in terms of prophylaxis against microbial invasion especially in rat tissue. Wound infection would seriously alter the results of the study. By using silver dressings it also allowed for the honey dressings to be compared in terms of tissue reaction, inflammation and toxicity.

In our study, all dressings seem to have good adherence to the wound bed. The H-honey dressing had the best score compared to the H-Ag and P-honey dressing (Table 3). The performance of the honey dressings used here was comparable to that of the control H-Ag dressing. Though there were significant differences in initial statistical analyses, the subsequent post hoc analyses showed no statistically significant differences between the honey dressings and the control dressing. The ease of dressing removal was slightly better in the H-honey and H-Ag dressings when compared to the P-honey dressing. However there were no significant differences between the control and the tualang honey dressings after post hoc

analysis with Mann Whitney tests. All dressing scores were moderate to good for this parameter. For other dressing and wound parameters assessed, all dressings scored similarly with a mean value above 2.3 for fluid accumulation, dryness of surrounding skin and amount of exudate. The tualang honey dressings performed as well as the positive control dressing and there was no statistical difference.

In terms of the healing rate and wound/scar contraction (Table 4), the initial mean of wound size ranged from 137-141 mm^2. The wound area steadily decreased and all wounds were fully healed by day 21. The rates of wound epithelization were inversely related to the wound area. There were no significant differences between the properties of tualang honey dressings and the control dressing. This equivalent performance result is important because of the considerably lower cost of honey dressings. The tualang honey dressings showed better stimulus for wound contracture when compared to the hydrofibre impregnated silver dressing. Wound contraction enables faster healing by reducing the area that needed to be covered by the process of epithelization. The wound contraction occurs in the proliferation phase of wound healing by myofibroblasts, which are the specialized contractile cells that pull the wound edges together leading to closure of the defect.

Histological examination revealed the expected pattern of inflammatory cell distribution according to phases of wound healing among the three dressings used in the study (Table 5). The tualang honey dressings were a good promoter of angiogenesis and were comparable to the control dressing. The honey dressings showed a lower inflammatory cell infiltration to the surrounding area, although they were not statistically significant from the control dressing. This result suggests that the tualang honey did not have an adverse effect on the wound condition and may be said to be less cytotoxic than the silver based dressing. Furthermore, the honey dressing did not inflict an excessive inflammatory stimulation. Inflammatory stimulation may have an adverse effect on wound healing and scar formation.

In vitro studies (Tonks *et al.,* 2003) have shown an increased release of the tumour necrosis factor-alpha, interleukin-1beta and interleukin-6 with several honey types and have suggested that the effect of honey on wound healing may be related to the stimulation of inflammatory cytokines from monocytes. These types of cells are known to have an important role in wound healing and are found in higher numbers in the wounds treated with tualang honey dressing.

The number of fibroblasts were relatively high in all groups at day four and then they rose slightly before they reached a plateau at day fourteen. As a result, the numbers of collagen fibres increased as the healing process continued. Skin appendages started to appear at the healed area at day 21 with all types of dressings used. Hair follicles and sebaceous glands were present in higher densities with the P-honey dressing compared to the other dressings used, but this result was not significant as the results observed were likely due to chance. Overall, the histological examinations of tualang honey dressings were comparable to the well-established modern dressing of silver impregnated hydrofibre dressing.

Conclusion

Tualang honey impregnated dressings used in this study had good wound healing and dressing properties. The honey impregnated dressings were comparable to the commercially established wound dressing material (silver impregnated hydrofibre) in term of adherence, ease of removal and fluid accumulation.

Histological examination of the wounds and scars showed that the wound healing progress proceeded in an orderly manner without over activation or prolongation of inflammatory processes. The wound healing process was comparable in all three types of dressing. Tualang honey impregnated dressings were as effective as silver impregnated hydrofibre dressing in terms of promoting wound healing and appeared to cause less tissue reaction.

The tualang honey dressing has a potential medicinal benefit, especially as a dressing for wound management at a lower cost. The results from this animal study are encouraging and provide evidence that tualang honey is effective in promoting wound healing and is a suitable dressing for full thickness wounds. A human study should be conducted to further investigate this evidence and assess whether there are any adverse effects for human use.

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