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Macroscopic Evaluation of Burn Wounds Healing Progress Treated with Different Types of Honey

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Abstract: The study was conducted to evaluate the macroscopic changes of burn wounds healing progress as a response to various types of honey applied topically. A total of 42 male, Sprague Dawley rats (weight 200-300 g) were used in this study. The animals were randomly divided into seven experimental groups consisted of 6 animals for each group. Macroscopic changes of the burn wounds healing progress were evaluated at day 0 post burned and subsequently at days 3, 7, 14, 21 and 28 post burned. To determine the rate of wound contraction the changes in the size of burn wounds were traced by measuring the diameter of the wound area both longitudinally and transversely across the body length using a ruler. The change in wound diameter at various time intervals was calculated as the percentage of wound area that had healed. The results obtained from this study indicated that topical application of Manuka and *Melaleuca* (Gelam) honeys significantly stimulated the rate of burn wound healing as assessed by increased in the rate of wound contraction and from the observation of gross appearances.

Key words: Macroscopic, honeys, burn wound healing, wound contraction

INTRODUCTION

During the past decade there has been a global interest in the use of traditional and complementary medicine. Most scientific and medical research has focused on herbal as well as aromatherapy products. In addition, a number of other natural occurring substances have been proved to show therapeutic promise. One such resource that was claimed to have curative value is honey. Honey has been used to treat a wide range of wounds of various aetiology including abscess, surgical wounds, ulcers and burns[1]. It was claimed that early Egyptians were the first to use honey as a component in the topical treatment of wounds as evidence from their writing in the Smith papyrus (1650BC)[2]. Thus, Zumla and Lulat[3] referred honey as ‘a remedy rediscovered’ due to the resurgence of its usage in modern professional medicine. Perhaps the rising interest in the use of alternative therapies is mainly due to the expanding problem of antibiotic resistance in bacteria or because some people are experiencing the sometimes side effect of many pharmaceuticals products[4].

One of the pivotal parameters in comparing the cosmetic effect of different treatment regimen is the visual appearance of the skin condition[5]. Observable changes occur in the physical appearance as well as in the physical characteristics of the skin due to the burn wound healing process. Thus, one of the fundamental parameters in comparing the efficacy of different treatment regimens in treating burn wound is the visual appearance of the skin condition. The dermal changes caused by diversity of injuries as well as the responses of these changes to various treatments have been addressed in several studies[6]. Furthermore, applying different topical regimens and stimulants to the burn wound in order to enhance the healing process have produced variable results.

According to Jackson[7], burn wound goes through a further series of appearances which sometimes enables the observer to guess within a few days the period since injury. Burn wound appearance has always been associated with the severity of the burn injury[8] and the appearance may also provide further information of the stage of healing process. Wound contraction is part of the normal process of healing and provides closure to the wound. Thus, observation of the visible appearances of the burn wound healing as well as measurement of wound contraction are reliable parameters to study burn wound healing. The present study was carried with the aim to evaluate the macroscopic changes of burn wounds healing progress as a response to various types of honey applied topically.

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MATERIALS AND METHODS

Animals and experimental design: A total of 42 male, Sprague-Dawley rats (weight 200-300 g) were used in this study. The animals were randomly divided into seven experimental groups with each group consisted of 6 animals. Group 1, 2, 3 and 4 were treated with *Melaleuca* spp. (Gelam) honey, *Cocos nucifera* spp. (Kelapa) honey, *Ananas comosus* spp. (Nenas) honey and *Durio zibethinus* spp. (Durian) honey, respectively. Group 5 and 6 were treated with Manuka honey and Silver Sulphadiazine (SSD) cream respectively, while Group 7 was left untreated and acts as control group. The progress of burn wound healing was recorded at days 0, 3, 7, 14, 21 and 28 post burned. The rats were kept in the animal unit at least one week prior to the experiment. The rats were given commercial pellet and water *ad libitum* throughout the study. The experimental protocol was approved by the Faculty's Ethic Committee.

Skin preparation: The skin preparation was done under general anaesthesia. The rats were anaesthetized with an intramuscular injection of ketamine (50 mg kg^{-1}) and xylazine (5 mg kg^{-1}) and the back and flank of both sides were shaved using razor blade. Since shaving procedure produced marked oedema of the skin, the prepared rats were left for twenty four hours before the burn was inflicted.

Thermal source: A method described by Kauffman *et al.*[9] was used with modification. Cylindrical aluminium templates (diameter, 2.5 cm; height, 3 cm; length of the handle, 24 cm; total weight 400 g) were heated in a waterbath for 3 h prior to injury at a constant temperature of 85°C. By this time, the templates surface temperature equaled that of the surrounding heating water. Five templates were heated concurrently and used alternately one for each injury and then were returned to the heating water to ensure maintenance of the desired temperature of the template surface. Approximately 5 min were elapsed between reusage of the templates.

Burn wound infliction: The burn was inflicted on the dorsum of the body between the last thoracic vertebra and the first sacrum. The rat was positioned on sternal recumbency. The anaesthetized rat was restrained and stretched on a metal board and location of the burn was marked between the last ribs and the horizontal line of the sacroiliac joints.

The heated and moistened template was applied at the right angles perpendicular to the dorsum of the rat according to the pre-marked location for a period of 5 sec using an analogue stopwatch. Minimal and constant pressure was applied to ensure a perfect contact between the template surface and the skin. The skin should be completely smooth so that sufficient contact and uniform pressure over the entire lesion was ensured.

Honey samples: Four selected monofloral *Apis cerana* honeys which produced by bees kept under different plantation were used in this study. Their floral sources were from *Melaleuca* spp. (Gelam) trees, *Cocos nucifera* spp. (Kelapa) trees, *Ananas comosus* spp. (Nenas) trees and *Durio zibethinus* spp. (Durian) trees. The honeys were named according to their floral sources. All the honeys were supplied by the Department of Agriculture, Malaysia. The Manuka honey was supplied by ZHR Technologies Sdn. Bhd., while Silver Sulphadiazine (SSD) cream was purchased from the Hospital Kuala Lumpur. All the honeys and SSD cream were irradiated at 25 kg using sinagama machine (Model JS8900) for sterilization purposes.

Mode of treatment: For all the treatment groups except in control group, the different type of honey in quantities of approximately 0.5 mL was applied topically onto the surface of each burn wounds. A thin layer of SSD cream was applied on the burn wound in the positive control group. The first treatment was applied approximately half an hour after burn infliction. The treatment groups received topical application twice a day until they were euthanased. The negative control group received identical burn and environmental exposure but no further treatment were given.

Macroscopic evaluation: After the burn infliction, gross changes of burn wounds were evaluated at day 0 post burned and subsequently at days 3, 7, 14, 21 and 28 post burned and the images were captured using a digital camera (Nikon Coolpix 4500) and the morphological evaluation such as appearance of the wound were recorded.

Rate of wound contraction: To determine the rate of wound contraction the changes in the size of burn wounds were traced by measuring the diameter of the burn size both longitudinally and transversely across the body length using a ruler. The data were recorded at day 0 post burned and subsequently at 3, 7, 14, 21 and 28 days post burned. The change in wound diameter at various time intervals was calculated as the percentage of wound area that had healed.

The results of wound contraction studies of all groups were statistically compared and p-value were calculated. At each interval, the wound margins were measured both longitudinally and transversely across the body length to calculate the non-healed area, which was then subtracted from the original wound area to obtain the healed area. The wound contraction percentage was determined using the following formula established by Baie and Sheikh[10]:

$$\%\ \textbf{wound contraction} = \frac{\textbf{Healed area}}{\textbf{Total area}} \times 100$$

Statistical analysis: Data were expressed as mean and standard deviation. Collected data were analyzed with two-way and one-way ANOVA test. p-value less than 0.05 were considered to be significant.

RESULTS

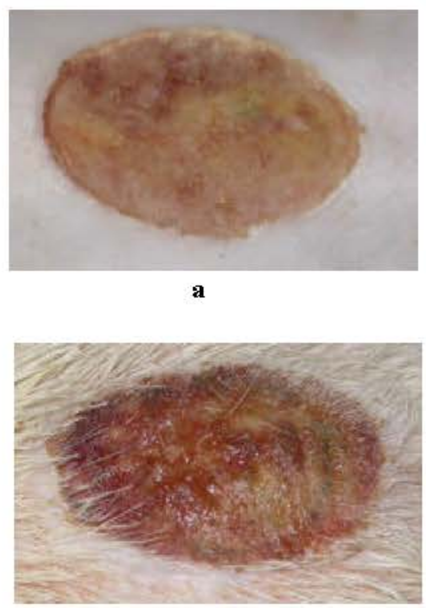
Macroscopic appearance: Immediately after infliction, the burned skin showed notable paleness with pearly white coloration and red rim around lesion in all experimental animals (Fig. 1). No macroscopic differences were observed between the experimental groups. By day 3 post burned, the burned skin started to exhibit intact, soft and supple yellow-brown discoloration in all groups of treatment and enlargement of the wound size from the initial appearance were observed (Fig. 2). Towards the end of the first week all burn wounds were covered with brownish thin scab. However, untreated control group and SSD cream treated wounds appeared to be covered with dry scab in contrast to honey treated wounds which were covered with moist scab (Fig. 3).

A marked improvement was seen in the appearance of the burn wounds after 14 days treated with honeys. On day 14, untreated control wounds were covered with stiff, intact, dark brown discoloured scab. However, the scabs formed by honey-treated wounds were moist as compared to untreated control group and SSD cream treated wounds. By day 21 post burned, scab in untreated control group still exhibited dry appearance with intact brown scab. Nonetheless, scabs covered the honey treated wounds as well as SSD cream treated groups started to slough off leaving behind minimal crust remnants in a predominantly epithelialized wounds with the wounds getting considerably smaller (Fig. 4). By day 28 post burned, honey treated wounds showed better sign of healing in comparison to untreated control and SSD cream treated wounds with the least scar was observed in Manuka treated wounds followed by *Ananas comosus* spp. (Nenas) and *Melaleuca* spp. (Gelam) honeys (Fig. 5).

Wound contraction: By day 3 post injury, there was a progressive of injury resulted in the enlargement of the burn size especially on the longitudinal measurement. Transverse measurement of the wounds showed no significant difference of the enlargement of wound area

Fig. 1: Burn wound appearance immediately after infliction. The wound appears pale with pearly white coloration and red rim around lesion

Fig. 2: Burn wound appearance of *Melaleuca* (Gelam) honey-treated group 3 days post-infliction. The burn size is enlarged as compared to day 0 post burn



a

b

Fig. 3: Burn wound appearance of a) control group and b) *Ananas Comosus* spp. (Nenas) treated group at 7 days post burn. Note that the control wound is covered by dry scab while the honey treated wound is covered with moist scab

Table 1: Longitudinal and transverse measurements of wound area as percentage of original wound size at day 3 post burns

	Percentage of wound progression (%)						
Measurement	Control	Nenas	Gelam	Durian	Kelapa	Manuka	SSD
Longitudinal	47.83 ± 12.61^{b}	41.21 ± 17.04^{ab}	27.23 ± 11.16^{a}	37.10 ± 8.06^{ab}	39.68 ± 18.44^{ab}	25.61 ± 8.08^{a}	40.38 ± 20.68^{ab}
Transverse	6.55 ± 13.44^{a}	8.28 ± 5.72^{a}	6.46 ± 3.18^{a}	4.43 ± 7.37^{a}	6.56 ± 3.65^{a}	1.36 ± 2.11^{a}	6.75 ± 5.52^{a}

^{a,b,c,d}Means with different superscript(s) within a row were significantly different at $p<0.05$

Table 2: Longitudinal measurements of wound contraction of the control and treated groups at different days as percentage of original wound size

	Percentage of wound contraction (%)						
Days post injury	Control	Nenas	Gelam	Durian	Kelapa	Manuka	SSD
7	$1.30\pm2.01^{a,w}$	$2.41\pm2.87^{a,w}$	$5.60\pm3.65^{a,w}$	$3.20\pm5.10^{a,w}$	$4.33\pm3.64^{a,w}$	$6.05\pm2.83^{a,w}$	$3.41\pm5.34^{a,w}$
14	$6.75\pm5.20^{a,wx}$	$6.58\pm0.040^{a,wx}$	$33.10\pm33.97^{ab,x}$	$14.46\pm13.32^{a,w}$	$17.08\pm8.37^{ab,wx}$	$32.86\pm14.22^{ab,x}$	$18.90\pm4.70^{ab,wx}$
21	$19.41\pm9.92^{a,x}$	$29.36\pm35.35^{a,xy}$	$56.10\pm9.50^{a,y}$	$43.51\pm44.29^{a,xy}$	$35.30\pm32.49^{a,x}$	$52.66\pm24.69^{a,y}$	$42.00\pm29.92^{a,xy}$
28	$46.48\pm27.10^{a,y}$	$64.16\pm23.89^{ab,z}$	$83.86\pm18.44^{b,y}$	$66.40\pm20.87^{ab,z}$	$71.16\pm31.87^{ab,y}$	$87.20\pm14.99^{b,z}$	$63.00\pm19.28^{ab,y}$

^{a,b,c,d}Means with different superscript(s) within a row were significantly different at $p<0.05$ due to treatment
^{w,x,y,z}Means with different superscript(s) within a column were significantly different at $p<0.05$ due to days post injury

Table 3: Transverse measurements of wound contraction of the control and treated groups at different days as percentage of original wound size

	Percentage of wound contraction (%)						
Days of injury	Control	Nenas	Gelam	Durian	Kelapa	Manuka	SSD
7	$9.50\pm7.36^{ab,w}$	$6.75\pm4.57^{ab,w}$	$8.58\pm4.60^{ab,w}$	$10.61\pm5.66^{b,w}$	$5.80\pm4.55^{ab,w}$	$7.61\pm6.33^{ab,w}$	$3.43\pm3.04^{a,w}$
14	$24.26\pm17.29^{ab,wx}$	$13.63\pm5.46^{a,w}$	$42.90\pm30.83^{b,x}$	$20.83\pm17.83^{ab,w}$	$17.13\pm2.30^{a,wx}$	$43.05\pm14.19^{b,x}$	$32.20\pm7.46^{ab,x}$
21	$38.10\pm19.97^{a,xy}$	$44.76\pm32.27^{a,x}$	$60.65\pm3.04^{a,y}$	$59.18\pm34.44^{a,x}$	$35.43\pm31.97^{a,x}$	$60.11\pm25.18^{a,y}$	$55.13\pm23.15^{a,y}$
28	$50.21\pm27.35^{a,y}$	$71.75\pm21.86^{ab,y}$	$87.50\pm15.36^{b,z}$	$77.18\pm14.02^{ab,x}$	$72.46\pm31.31^{ab,y}$	$90.71\pm10.47^{b,z}$	$69.60\pm16.31^{ab,y}$

^{a,b,c,d}Means with different superscript(s) within a row were significantly different at $p<0.05$ due to treatment
^{w,x,y,z}Means with different superscript(s) within a column were significantly different at $p<0.05$ due to days post injury

among the experimental groups. However, longitudinal measurement resulted in significantly lower progression of injury in Manuka honey (25.61±8.08) and *Melaleuca* (Gelam) honey (27.23±11.16) treated wounds as compared to control group (Table 1).

On day 7 post burned, longitudinal measurements of the burn wounds area showed no significant different among the treated wounds and untreated control wounds. However, the rate of contraction was higher in wounds treated with Manuka honey (6.05±2.83) as compared to other experimental groups although it was not statistically significant (Table 2). On the other hand, transverse measurement exhibited higher percentage of wound contraction in *Durio zibethinus* (Durian) honey treated wounds (10.61±5.66) (Table 3). Whilst SSD cream treated wounds showed significantly lower contraction rate (3.43±3.04) as compared to other groups. It was observed that there was a significant increased of wound contraction from day 7 to day 14 post wounding in *Melaleuca* (Gelam) honey and Manuka honey treated wounds for both measurements ($p<0.05$).

At 14 days post burned, longitudinal measurement of wound contraction of *Melaleuca* (Gelam) honey treated wounds (33.10±33.97) and Manuka honey treated wounds (32.86±14.22) exhibited higher percentage of wound contraction as compared to other experimental groups (Table 2). Nonetheless, the rate of wound contraction does not differ significantly among experimental groups. Transverse measurement of wound contraction demonstrated that Manuka honey treated wounds showed the highest percentage of wound contraction (43.05±14.19) followed by *Melaleuca* (Gelam) honey treated wounds (42.90±30.83). It was observed that at 14 days post burned the percentage of wound contraction on transverse measurement was significantly higher in Manuka honey treated wounds (43.05±14.19) as compared to *Ananas comosus* (Nenas) honey (13.63±5.46) and *Cocos nucifera* (Kelapa) honey (17.13±2.30) treated wounds (Table 3). Both measurements demonstrated significant increased from day 14 to day 21 post injury in *Melaleuca* (Gelam) honey, *Durio zibethinus* (Durian) honey and Manuka honey treated wounds ($p<0.05$).

At 21 days post burned, both longitudinal and transverse measurements of the burn wounds area showed no significant difference among the treated wounds and untreated control wounds. Nonetheless, *Melaleuca* (Gelam) honey treated wounds demonstrated the highest percentage of wound contraction (56.10±9.50 and 60.65±3.04 for longitudinal and transverse measurements respectively). While untreated control group showed the lowest percentage of wound contraction (19.41±9.92 and 38.10±19.97 for longitudinal and transverse measurements, respectively) (Table 2). It was observed that *Ananas comosus* (Nenas) honey, *Cocos nucifera* (Kelapa) honey and Manuka honey treated wounds exhibited significant increased in wound contraction from day 21 to day 28 post wounding ($p<0.05$).

a

b

Fig. 4: Burn wound appearance of a) control group and b) *Cocos Nucifera* spp. (Kelapa) treated wound at day 21 post burned. Note that the scab in untreated control group still exhibited dry appearance with intact brown scab while the scabs covered the honey treated wounds has slough and the wound getting considerably smaller

a

b

Fig. 5: Burn wound appearance of a) control group and b) *Melaleuca* (Gelam) honey treated wound at 28 days post burn. Note that honey treated wounds showed better sign of healing as compared to untreated control wound

By day 28 post burned, wound contraction had progressed with the wounds area getting considerably smaller in honey treated wounds as compared to untreated control wounds. Manuka honey treated wounds demonstrated the highest percentage of wound contraction on both longitudinal (87.20±14.99) and transverse (90.71±10.47) measurements. Followed by *Melaleuca* (Gelam) honey treated wounds with 83.86±18.44 on longitudinal measurement and 87.50±15.36 on transverse measurement. Statistically, both Manuka honey and *Melaleuca* (Gelam) honey treated animals showed significant difference as compared to untreated control groups ($p<0.05$). However, the amounts of percentage of wound contraction for both groups were not significantly different when compared to other treated groups (Table 2).

In addition it was observed that Manuka honey treated groups demonstrated significant increased from day 7 to 14 to 21 to 28 post injuries for both measurements.

DISCUSSION

Wound healing is a complex process which results in wound contraction and closure of wound as well as re-establishment of a functional barrier[11]. The repair of wounds involves different phases including contraction, formation of epithelialisation and fibrosis[12]. Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin[13]. In this experiment, progressive injuries of burn wounds were observed at early stage from the time of initial infliction until day 3 post burned. This is in agreement with Knabl *et al.*[14] who stated that demarcation of the necrosis take place during the first days post burn and reached a stable border of necrosis after three days. Progressive skin necrosis after burn trauma is a frequent occurrence[15] with the progression of zone of stasis becoming part of the enlarged zone of coagulation[16]. It has been shown that the occurrence of progressive tissue injury is related to an excessive inflammatory response at the wound site and that necrotic tissue is one of the main factors leading to excessive inflammatory response[17]. Clearly, the presence of necrotic tissue is one of the negative factors leading to wound progressive damage at early stages after thermal injury. Based on experimental work with local burn models, free radicals and neutrophils adherence have been found responsible for this progressive tissue injury and further necrosis[18].

The present study demonstrated that topical applications of Manuka and *Melaleuca* (Gelam) honeys have better control of the progression of zone of stasis in the wound with the extent of skin damage was lower as opposed to other experimental groups. It could be inferred that these two types of honey may have higher antioxidant properties which helped in modulating the free radicals that present at the wound site, thus reducing the progressive thermal injury. Aljadi and Kamaruddin[19] reported that honey has antioxidative and radical scavenging properties, which are mainly due to its phenolic content. Honeys from different floral sources were assumed to have different total phenolic contents[19], thus contributed to the different antioxidant activity and therapeutic effects. The results obtained in this study suggested that Manuka and *Melaleuca* (Gelam) honey might contained higher total phenolic contents, hence the less progression of thermal injury of the zone of stasis.

During this study, it was observed that contractions of the wounds were evident in all experimental groups, especially at later time points (21-28 days). All the treatment groups showed maximum rates of contraction at the period between 21 to 28 days post burned during the course of study. However, in comparison, the wound contracting ability of topical application of *Melaleuca* (Gelam) honey and Manuka honey was higher than other experimental groups. The wound contraction ability may be attributed to the presents of myofibroblasts in the granulation tissues[20]. The accelerated onset of wound contraction in Manuka and *Melaleuca* (Gelam) honey treated wounds, judged by qualitative comparison of photographic records and percentage of wound contraction, suggested either that their granulation tissue contained a more effective or greater number of contractile myofibroblasts than other groups as in agreement with the study conducted by Aljady *et al.*[21].

Contraction reduces the size of the tissue defect and limits the volume of scar tissue, while encouraging repair by intussusceptive growth of the adjacent, undamaged normal skin[20]. The energy required for wound contraction is generated by cellular activity[22]. Manuka and *Melaleuca* (Gelam) honeys treated wounds showed an increased in the rate of wound contraction, leading to quicker healing as confirmed by the increased healed area when compared to untreated control groups. Honey is known to have high concentration of sugar and high level of amino acids such as glycine, praline and methionine, which are favourable for early formation of granulation tissues that helped in faster wound healing[23]. Topical application of honey at the wound site produced significant wound healing, which also may be due to the honey's osmotic effect that creates moist environment thus provide oxygenation and nutrification for the traumatized tissue through the flow of lymph that is induced[24].

The greater the amount of wound contraction during repair, the less scar tissue deposited[25]. Normal skin is both mechanically and cosmetically superior to scar tissue, thus early contractions are desirable, in addition that it does not produce local deformation[20]. Fibroblasts within the granulation tissue are also responsible for wound contraction[22]. Wound contraction occurs as a result of an interaction between fibroblast locomotion and collagen reorganization[21]. Aljady and Kamaruddin[19] reported that wound contraction might be enhanced by stimulating fibroblasia and collagen deposition as well as by providing the energy needed for this contractile. Thus, in the present study, Manuka and *Melaleuca* (Gelam) honey seemed to have additional influences on various stages of dermal repair resulted with faster healing. In addition, the scab which formed to cover the wound site provided limited protection from external contamination, maintains internal homeostasis and provides a surface beneath which epithelial cell migration and movement of the wound edges can occur[22]. During this study, the scabs that covered honey treated animals were moist in comparison to the untreated control group and SSD cream treated animals. This observation could be attributed to the moist milieu created by honey, due to its viscosity[26].

In conclusion, the observations and results obtained from this study indicated that topical application of Manuka and *Melaleuca* (Gelam) honeys significantly stimulated the rate of burn wound healing as assessed by increased in the rate of wound contraction and from the observation of gross appearances. The differences in the healing activity of different honeys are likely to be attributed to the different floral sources. However, further experiments are needed to test and re-confirmed the present study. *In vitro* study should be carried out in order to obtain a firmer result.

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