4.5 DISCUSSIONS

Many researches on the efficacy of the topical application of honey in wound management had been done (Stephen et al., 2009). However, the effects of Malaysian honey have not been well established neither; macroscopically nor microscopically. Hence, this chapter of study was done to evaluate the wound healing process of the normal wound and also the efficacy of the two selected Malaysia honey (Gelam honey and Nenas honey) by histological approach.

Results from the microscopic evaluation of this study showed that topical application of both Malaysian honey (Gelam honey and Nenas honey) helped to accelerate wound healing process. Summary of the results obtained from this study are presented in Table 4.11.

Table 4.11: Summary of the results obtained from the observation of this study.

	Group NO (No Dressing)	Group SA (Saline)	Group IN (Intrasite)	Group GE (Gelam)	Group NE (Nenas)
Abundant Macrophages	Day 5	Day 5	Day 5	Day 5	Day 5
Inflammation Cleared	Day 10	Day 10	Day 5	Day 5	Day 5
Epithelialization Started	Day 5	Day 1	Day 1	Day 1	Day 1
Epithelialization Completed	>Day 15	> Day 15	Day 15	Day 15	Day 15
Angiogenesis started	Day 10	Day 5	Day 5	Day 5	Day 5
Angiogenesis Ceased	Day 15	Day 15	Day 10	Day 10	Day 10
Presence of Scab	Day 15	Day 15	Day 10	Day 10	Day 10
Inflammation Phase	Day 15	Day 10	Day 5	Day 5	Day 5
Proliferation Phase	Day 10	Day 10	Day 5	Day 5	Day 5
Remodeling Phase	Day 15	Day 15	Day 10	Day 10	Day 10

In Group NO (No dressing) and Group SA (Saline), the wounds were still in the inflammation phase (the first phase of wound healing) on Day 10 of treatments. The prolonged inflammatory phase in the wound for these groups could be due to infection or contamination of wound site. This is then lead to the delay of the wound healing process. Scabs were still attached on the surface of wounds on Day 15 of treatments. Epithelialization started from the beginning of the wound healing process but

prolonged until Day 15 of treatments in both of the groups. The newly formed epidermis was thin and incomplete, thus it was expected to be very vulnerable. Angiogenesis started late in Group NO (No dressing) and Group SA (Saline) and it ceased only after Day 15 of treatments. Thus, the wound healing process in Group NO (No dressing) and Group SA (Saline) were generally delayed compared to the other groups.

Wounds in the three treatment groups showed positive results. The wounds treated accordingly with the three dressings (Intrasite gel, Gelam honey and Nenas honey) showed positive result in wound healing starting from Day 5 of treatments. As expected, Intrasite gel treatment helped in clearing the inflammation of the wounds. Thus, it accelerated the process of the proliferation. The proliferation phase started on Day 5 of treatments in Group IN (Intrasite). This was shown by the increase fibroblasts on Day 5 of treatments (Figure 4.31). Besides, angiogenesis level was also increased on Day 5 of treatments of the wound in Group IN (Intrasite) (Figure 4.30). The epithelialization was completed on Day 15 of treatments in Group IN (Intrasite) when the scabs were fully detached (Figure 4.68).

In both honey treatments (Group GE and Group NE), the results obtained were similar to Group IN (Intrasite). Both honey helped in reducing the inflammatory phases at the early stage of the wound healing process. Proliferation phase started on Day 5 of treatments and the remodeling phase was observed on Day 10 of treatments. Both honey treatments showed similar results, where by epithelialization was completed on Day 15 of treatments. However, epithelial layer in the wounds of Group NE (Nenas) was thinner compared to wound of Group GE (Gelam).

On Day 1 of treatments, demarcation line which formed in the wounds in all the treatment groups (Figure 4.10) showed that the migration of the inflammatory cells to

the wound area occurred. Result showed that the inflammatory phase started on Day 1 of treatments on the wounds treated with wound dressings (Intrasite gel, Gelam honey and Nenas honey). These treatments accelerated the process of cells migration to the wound site at the beginning stage of wound healing. This would indirectly lead to acceleration of the healing process due debris and bacteria being phagocytized(Torre and Chambers, 2008). The area of wounds was faintly stained in yellowish color in the VE stains (e.g. referred to Figure 4.18). This indicated wound area of all treatments groups were filled with exudates (e.g. pus). Collagen and fibroblast level remained low at this stage of healing.

The anti-inflammatory effect of honey has been observed microscopically in several animal studies (Aljady 2003). The results obtained from this study showed that both types of honey also reduced the inflammation in wound healing process, with Nenas honey showing the greater efficacy. There were also studies which reported that honey helped in clearing infections and protected the wound from being infected (Norimal et al., 2007). Besides that, honey also acts as an antibacterial barrier to the wounds and this prevents inflammation and also infection of the wound to occur (Aljady, 2003). Thus, in concordance to the present study, level of macrophages started to reduce from Day 5 of treatments in the honey treated groups (Figure 4.5). Suguna et al. (1992) reported that less neutrophilic infiltration found in honey treated wound. This present study showed that honey accelerated the inflammatory reaction and initiatds the healing process in the early phases of healing. Early attenuation of the inflammation reaction was found in honey treated wound and Intrasite gel treated wound. There were reparative activities found in the honey treated wounds compared to Group NO (No dressing) and Group SA (Saline).

Many literatures showed that there are features related to the granulation tissue in the excisional wound healing that could be relied on as parameters of dermal healing

(Aljady, 2003). Combination of cellular elements seen in the granulation tissue formation on proliferation phase included fibroblasts, inflammatory cells and also new capillaries (Thomas Romo III et al., 2008). Fibroblast is the predominant cell type in the granulation tissue. They are responsible for the production of the structural proteins used during tissue reconstruction (Baie and Sheikh, 2000).

In this study, results of the microscopic evaluation demonstrated that honey treatment, especially Nenas honey enhanced the process of fibroplasia. Fibroplasia was increased drastically in Day 5 of treatments and decreased in Day 10 of treatments in the wounds treated topically with Nenas honey (Figure 4.8). Fibroplasia in Group NE (Nenas) was initiated earlier than the others (Figure 4.38). Past study reported that rapid fibroblastic and angioblastic activities were observed in honey applied wounds (Gupta et al., 1992) and honey stimulated fibroplasia *in vitro* (Aljady, 2003). Hence, results of the current study was in agreement with previous studies that formation of fibroblasts and angiogenesis were greater in honey treated group on Day 7 of treatments (El-Banby et al.,1989; Nisbet et al., 2010). Both fibroplasia and angiogenesis are two fundamental process in the wound healing process (Regan and Barbul, 1994).

Fibroblasts cell which is responsible for initiating angiogenesis, epithelialization and collagen formation (Gabrie et al., 2009) pre-dominated the wound area. Microscopic evaluation in this studys showed angiogenesis level started to increase since the beginning of the wound healing process. There was a gradual increase in the number of blood vessels found by Day 5 of treatments in this study, especially to the wound treated with wound dressings (Intrasite gel, Gelam honey and Nenas honey). The number of blood vessels found in wound treated with both honey (Gelam honey and Nenas honey) was greater compared to the Intrasite gel treatment group (Figure 4.7). This indicated honey treatments helped to accelerate the wound healing process by initiating the angiogenesis process as early as Day 5 of treatments. Big blood vessels

were found at the wound area (Figure 4.31 and Figure 4.38) in wounds of both honey treatments. The increased in the angiogenesis process will accelerated the healing process as the newly formed blood vessels acted as the blood supply to the newly formed granulation tissue (Mercandetti and Cohen, 2008).

Microscopic evaluation showed that the angiogenesis process in Group NO (No dressing) and Group SA (Saline) did not increase much by Day 5 of treatments. Group IN (Intrasite) showed stagnant results of the number of blood vessels in the wound site from Day 5 to Day 10 of treatments. Theses proved that the wounds in these three experimental groups entered remodeling phase later than the honey treated groups (Group GE and Group NE). In addition, the size of blood vessels in the honey treated groups (Figure 4.35 and Figure 4.40) was larger compared to the one in Intrasite gel treated group (Figure 4.30). Angiogenesis process ceased after the wound was fully recovered. Thus, when the number and sizes of the blood vessels decreased and disappeared respectively, from the surface of wound this indicated that the healing process entered the last phase of wound healing (the remodeling phase) (Figure 4.7). Both honey treated groups entered the remodeling phase earlier compared to the other treatment groups.

Microscopic evaluation of this study also showed that epithelial regeneration appeared more significantly in wound under honey treatments especially in Group GE (Gelam). This might be due to the honey dressing (Gelam honey and Nenas honey) having properties that enhanced the rate of various phases of the wound healing process. For example, increase the fibroblast level could lead to the collagen formation and angiogenesis. Results obtained from this study showed that both honey treatments achieved complete epithelialization on Day 15 of treatments. Gelam honey seemed better in accelerating the epithelialization process. As for the Intrasite gel treatments, it might give the same results of epithelialization if the scab on the surface was detached

completely. Intrasite gel treatment is the modern wound dressings that commonly used in prescription in hospital. It gave positive result to the wounds similar to the honey treatments, but it caused some side effects, such as hard and adhesive dry scab being formed on the wounds. This might then lead to the delay in the wound healing process.

Besides that, honey also contains high levels of glycine, arginine and proline which are essential in collagen formation and deposition (Aljady, 2003). Other than that, the acceleration of healing by honey treatment might also be due to the general characteristic of honey, such as hydroscopic properties, antibacterial properties, antioxidant properties, nutritional properties and production of hydrogen peroxide. For example, the low pH level of honey creates an unfavorable environment for bacterial growth (Molan, 2000). Honey also contains bee pollen enzymes that can stimulate new tissue growth (Nisbet et al., 2010). Honey may contain medicinal compounds such as essential oil, flavanoids and terpenes depending on the origin of the nectar.

Although the capacity of the skin to heal itself is large, restrictions such as the dryness of the skin could be a factor for the unhealed wound (Salami et al., 2006). Keeping the surface of the wound in moist condition could be one of the factors that enhanced the healing process. Efem (1988) and Gupta et al. (1992) reported that epithelialization was faster in honey treated groups. The viscosity of honey possibly promotes rapid epithelialization (Subramanyam, 1998). In agreement with previous animal study on Malaysian honey (Aljady et al., 2000), there was enhancement of the epithelialization process found in honey treated groups in this study. This enhancement of epithelialization by honey might be contributed by the viscosity of honey. This characteristic of honey possibly provided the wound with a moist healing environment, which promotes healing process. The suitable environment could promote cells migration and regeneration of epithelial cells. Honey dressings also helped to form a clean wound surface with thin scab, which was easily detached from the wound and

this would also favor the healing process. Although the Intrasite gel provided the similar results in wound healing, this dressing did not provide the moist environment that favors the wound healing process. The wound healing process could be delayed due to the late detachment of the thick and hard scab.

Other factors such as the increased hyaluronic acid could also increase the rate of epithelialization. Hyaluronic acid could create a moist wound environment and maintain tissue hydration (Ballard and Bazter, 2000). Low pH of honey could also accelerate the rate of epithelialization. Honey treatment was also said to be capable of significantly increasing the protein synthesis and cell proliferation in newly formed granulation tissues, as reflected by protein and DNA levels (Aljady et al, 2000).

Collagen fibers play an important role in maintaining the structural integrity and healing wound (Ukong et al., 2008). The results of the present study showed that collagen level in the granulation tissue was greater compared to the other groups, except Intrasite gel treated group. The previous study done by Aljady (2003) and Chithra et al. (1989) also showed that the collagen increased in wound healing could be the most important characteristic in wound healing (Rao et al., 2007). Therefore, many studies were done to quantify the amount of collagen changes and its orientation during the wound healing process. For this study, this wound healing characteristic was focused in with the simplest histological way, by using histological approach under light microscopy.

Suguna et al. (1992) also reported that honey accelerated the synthesis and maturation of collagen and resulted in the increased tensile strength of the healed skin. In the rat model, Rozaini et al (2005) proved that honey treatment increased collagen content in the wound and enhanced stabilization of fibers.

4.6 CONCLUSION

Honey plays a positive role in wound healing process. Gelam honey and Nenas honey produced similar effects in enhancing the wound healing activity. Both provided a clean and moist environment for healing. The application of honey on wound enhanced epithelialization, fibroplasias, angiogenesis, which contribute to the wound healing process. Results in this study showed that Nenas honey gave slightly better result in anti-inflammatory while Gelam honey showed better result in accelerating the process of epithelialization. Both honey treatments generally accelerated the wound healing process. As showed in the results obtained from the microscopic evaluations, topical application of pure honey on wounds could be a potential therapy for wound treatments.