3.5 DISCUSSIONS

Wound healing is a complicated and complex process that results in repair and regeneration of injured tissue, which involved multitudes level of cells and event (Moon and Crabtree, 2003; Braiman-Wiksman, 2007). This complicated healing process could be affected by several factors, both intrinsic and extrinsic factors (Rozaini et al., 2005). The intrinsic factors included the underlying pathophysiological factors; factors are those factors related to the medical status or physiological features of the patients, which would affect the skin integrity or the rate of wound healing. These factors include age, chronic disease, oxygenation and natural immunosuppressant in the body system. As for extrinsic factors, they are the environmental influences. The extrinsic factors involved those sources found in the environment such as medication, nutrition, infection, irradiation and chemotherapy. All of these factors might contribute to the delay of the healing process (Aljady et al., 2000; Aljady, 2003; Rozaini et al., 2005; Friedrich, 2010).

There were several reports about the role of nutritional support for wound healing (Percival, 1997; Mackay and Miller, 2003). Healthy nutrition is important in promoting wound healing. It is essential to prevent the complication associated with injuries, such as infections, keloids and weight loss (Friedrich, 2010). One of the main factors that lead to the non-healing of wounds was the problem of malnutrition faced by patients. This could lead to the problem of weight loss, protein energy malnutrition (PEM) and physiological stress related to the underlying illness and indirectly causing the challenge to the wound healing process (Mackay and Miller, 2003).

The results obtained in this animal study showed that the body weight (g) of the rats dropped in the beginning of the wound healing process (Day 1 to Day 5 of treatments). This problem might be caused by the loss of appetite of the rats. The pain

caused by the wounds might lead to the loss of appetite and indirectly lead to the decrease in food intake. Thus, the newly made wound status was indirectly related to the initial decrease of body weight. It is hard for the body to achieve the usual level of nutrient due to the loss of appetite. In addition, the reduction of appetite at the beginning in rats might be caused by the side effect of chemicals in anesthesia. Altered level of consciousness, poor appetite and compromised of blood circulation are the side effects of anesthesia (Tomlison and Ferguson, 2003).

A successful wound healing process requires adequate blood and nutrients supply to the wound site (Mackay and Miller, 2003). Most nutrients obtained from the diet are channeled into the wound sites and contribute to the wound healing process. Thus, the health and nutritional status of the rat might influence the outcome of the healing process. Any injury to the body might also lead to the increase in body's metabolic rates. Energy expenditures in the body may rise by 10% to 50% to support the metabolic workload for wound healing process, such as new tissue formation and proliferation (Omerbegovic et al., 2003). During injuries, the rats might become hyper metabolic. They might require a higher level of calories and protein to supply the necessary nutrients. Thus, it would contribute to the rapid weight loss.

The decrease of body weight (g) in the experimental animals might also be due to underlying stress after the wound creation. The pain caused by the excisions or wound might lead to the prolonged stress, which then lead to the catabolism where there will be a progressive loss in lean body mass (LBM). The results from this animal study showed that rats were under stress during the early stage of wound healing process. Patients who are under stress might need more calories and are also weak in their immune system. Stress hormones such as cortisol and catecholamines which could indirectly caused the lost of body weight are also released (Friedrich, 2010).

Protein energy malnutrition (PEM) might also contribute to the decrease in body weight (g) of the rats in the early stage of healing process. The intake of protein and calories of the rats is not enough due to the loss of appetite. Thus, the body used the LBM to meet the calorie needed for wound healing process. It then resulted in the loss of LBM and directly leads to the body weight loss. As an effect of PEM during wound healing, the calories and proteins needed for wound healing compete with that need to meet the body's basic needs. If these problems persist, they could lead to the delay of wound healing.

From Day 5 of treatments onwards, when the wound started to recover, the pain caused by the wound reduced, so as the stress caused by the pain. The rats were back to their normal daily activities. The intake of nutrients channeled to the wound sites for wound healing process also started to reduce. Thus, there was no competition between the nutrients to be used for body's basic need and for wound healing process; the body weight of the rats started to increase.

On Day 15 of treatments, body weight of the rats exceeded the original body weight because most of the wounds were healed. No extra nutrients were needed for the wound healing process at this time point. The rats were no longer under stress. Thus, the body weight of the rats increased as all nutrients consumed contributed to body's basic need.

Table 3.20: Summary of the results obtained from the animal study (n=6).

	NO	SA	IN	GE	NE
Healing Duration (Days)	16.67 ±0.80	15.83±0.40	13.00±0.37	13.17±0.48	13.00±0.51
Longitudinal Diameter (cm) ; Day 15	1.40±0.21	1.13±0.16	0.87±0.17	1.30±0.14	1.15±0.09
Transverse Diameter (cm); On Day 15	0.67±0.23	0.38±0.07	0.45±0.10	0.38±0.13	0.25±0.03
Wound Area (cm^2)	0.46±0.15	0.25±0.04	0.29±0.25	0.25±0.05	0.21±0.03
Longitudinal Contraction (%)	34.89±0.21	42.94±6.05	62.75±6.67	38.48±5.58	45.26±3.84
Transverse Contraction (%)	80.89±3.13	80.89±3.13	79.11±4.19	80.24±6.39	86.64±1.66
Wound area Contraction (%)	88.54±3.72	93.76±0.99	93.27±1.02	93.73±1.22	94.38±0.69

Wound healing is a time consuming process that involved three different phases; inflammatory phase, proliferation phase and remodeling phase. Even with the existence of the advance technology in modern therapy of wound management, the problems faced in wound healing still remained a concerned among the researchers. Several studies showed that honey dressing could provide an optimal healing environment which could accelerate the healing process (Moon and Crabtree, 2003). Although there are numerous wound dressing materials for the purpose of accelerating the wound healing process, the unwanted side effects caused by their applications lead to the increased interest in wound research using natural products (Moon and Crabtree, 2003). Honey is one of the rediscovered natural products found to be useful in wound healing process. Thus, the two Malaysian honey (Gelam honey and Nenas honey) were chosen as the wound dressing used in this animal study.

The results of this study found that application of wound dressing involving Intrasite Gel, Gelam honey and Nenas honey had significantly accelerated the wound healing process. The wound dressings used in this study showed similar result in which

the wounds healed around 13 days of treatments. Most wound dressing would promote and shorten the duration of wound healing process by providing the wound with an optimal healing environment that include pain relief, protections or physical barrier, moist environment and removal of debris (Moon and Crabtree, 2003). The wound dressings used in this study provided all the above mentioned characteristics.

Wound healing is a process of repair and regeneration of injured tissue that involved wound contraction and closure of wounds. This process included contraction, reepithelialization and fibroplasias (Khana et al. 2002). The present study demonstrated that the size of wound diameter and wound area in all treatment groups decreased from Day 1 to Day 15 of treatments. Intrasite gel treatment had significantly reduced the longitudinal wound diameter on Day 15 of treatments. While the smallest transverse wound diameter was found in the Nenas honey treatment. The size of wound diameter treated with Nenas honey was smaller compared even to those in Gelam honey treatment and Intrasite gel treatment.

The wound size was found to decrease from Day 1 to Day 15 of treatments. Gelam honey and Nenas honey treatments produced similar result in decreasing the wound area. By Day 15 of treatments, the wounds sizes are the smallest.

Wound contraction is an essential process in wound healing that would lead to wound closure. Thus, wound contraction had become a reliable parameter assessed through macroscopic evaluation for wound healing (Gal et al., 2005). The results of this study revealed that the greater the wound contractions, the smaller was the wound size (diameter and area) and the lesser the scar formation. The decrease in the wound diameter was due to the wound contraction. Thus, wound contraction had become an important and more accurate parameter in wound assessment compared to the measurement of wound size.

During the beginning of the healing process (Day 1 to Day 5 of treatments), lengthening of transverse wound diameter was observed in all the treatment groups. The phenomenon of the lengthening of transverse wound diameter rather than the contractions might be due to the behavior of the rats and skin elasticity. This is in agreement to the report from Braiman-Wisksman et al. (2007) which reported that after 3 hours of the wounding based on skin elasticity and behavior of incision becomes an elliptical, tear shaped wound throughout all layers of skin.

Wound contraction is one of the key features and predominant event in the wound healing process as it accelerated the wound healing process and minimized the size of wounds. This study also demonstrated that the contraction rate of the wound, as reflected by the longitudinal and transverse diameters as well as wound area, was seen to be greater in Group NE (Nenas) compared to the Group GE (Gelam). The treatment groups involving Intrasite gel, Gelam honey, and Nenas honey showed the effect of increasing rate of wound contraction after treatment compared to Group NO (No dressing) and Group SA (Saline).

The usefulness of honey in wound management have been previously reported (Medhi, 2008; Khoo et al., 2010). Different types of honey have been used in many animal experiments to show acceleration in wound healing and its antibacterial properties (Khoo et al., 2010). There were also studies on the application of Manuka honey to accelerate healing of acute wounds, chronic ulcers, burns, MRSA infections and infected wounds (Molan, 2001). According to Aljady et al. (2000) and Norimah et al. (2005), honey accelerated the wound healing process by enhancing the wound contractions. Wounds treated with honey were smaller and epithelialization from the periphery of the wound edge was increased. Honey might stimulate wound contraction

by providing the energy needed for contractile activity. It also enhanced the deposition of fibroblast and collagen, which contributes to healing (Aljady, 2000).

Wound contraction caused by the myofibroblasts is important for tissue contraction. Honey enhances the rate of wound contraction by providing the energy needed for contractile activity of the myofibroblast. Besides that, the low pH level of honey also helps in facilitating the contraction of wound because the acidic media could enhance myofibroblast contraction (Mohammad et al., 1998). This study had shown that the greater the wound contraction, the lesser the scar formation which is equivalent to the report by Medhi et al., (2008).

Honey also accelerated the wound healing process due to its low pH level and by exerting the antibacterial activity. The two types of honey used in the study have high antioxidant properties, which might contribute to modulating the free radicals in wound sites. Previous study done by Aljady et al. (2000) reported that honey has antioxidant and free radical scavenging properties which is caused by their phenolic content.

Although both honey treatments in this study showed positive effects, results obtained in both honey treatments were slightly different. Group NE (Nenas) showed slightly better results compared to the Gelam honey group. This could be due to the fact that the honey from different sources had different phenolic content (Aljady and Kamaruddin, 2004). Nenas honey might contain higher phenolic content compared to Gelam honey used, hence the efficacy of Nenas honey treatment was slightly better in this animal study.

In this study, the wound contraction was observed to be evident in all groups. Application of Intrasite gel and saline treatment in this study also stimulated wound

contraction. This might be due to both of these treatments provided the wound with moist environment. Moist environment is important in stimulating wound contraction in wound healing.

Maximum rate of contraction was found on Day 15 of treatments in all groups. This wound contraction activity might be attributed to the presence of myofibroblast in the granulation tissue (Rozaini et al., 2005). Thus, the greater rate of contraction in honey treatment groups suggested that honey stimulated the formation of granulation tissue which contained greater amount of myofibroblast (Aljady et al, 2000; Rozaini et al., 2005).

Wound contraction reduced the size of the wound and also limited the volume of the scar tissue. This could encourage the repair process by initiating the growth of the surrounding undamaged normal tissue (Rozaini et al., 2005). The increase in rate of wound contraction in honey treatments lead to a quicker healing as confirmed by the increase in healed area as compared to the Group NO (No dressing) and Group SA (Saline). The high concentration of sugars and amino acids contributed to the early formation of granulation tissue that helps in accelerating wound healing (Kumar et al., 1993).

Other than that, the acceleration of wound healing process caused by topical application of honey might also be due to the osmotic effect of honey. This characteristic of honey contributed to the moist environment to the wound healing process. Thus, it provided oxygenation and nitrification for the traumatized tissue through the flow of the lymph (Molan, 2001 and Aljady, 2003).

The overall results from the present study showed that wound dressings (Intrasite gel, Gelam honey, and Nenas honey) reduced the amount of exudates from

Day 5 of treatments onwards. The types of exudates changed from bloody to serous in the treatments groups from Day 5 of treatments onwards. Other than that, appearance of wound bed changed from unhealthy (bloody) to healthy (pink). The wounds in Group NO (No dressing) took longer time to heal due to the mild infection during the beginning of the wound healing. The reduced exudates in the honey treatment group might be caused by the hyperosmolarity characteristic of honey. This characteristic might also be one of the causes of the rapid absorption of oedema fluid from the soggy weeping wound (Subramanyam, 1998).

This present study also showed that scab formation honey treatments group was thinner than other groups from Day 5 of treatments onwards. This result is equivalent to the previous study done by Aljady (2003), where the scabs formed in honey treatment groups were thinner and not dry as seen in the control groups. Thinner scab formation might be due to the high viscosity of honey and its high sugar content that could provide the wounds with a moist environment (Aljady, 2003; Khoo et al., 2010). Thinner scab formation in the wound formed a smaller barrier for epithelialization to occur and thus accelerated the healing process. This study demonstrated that the topical application of honey, whether it was Gelam honey or Nenas honey reduced the hard, intact, dark brown scab in wound healing. It appeared to be clean and healthy. Besides that, the scab detached easily and the scar appeared to be thinner in both honey treated groups.

From this study, the results showed that Intrasite gel lead to production of hard scab on the wounds. The detachment of the scab was slower compared to the honey treatment group. Thus, the wound healing process might be affected due to the hard and thick scab formed on the surface of the wound. As the scab was thicker, the barrier of

epithelialization of the wound was larger. This might had affected the efficacy of wound healing in the Intrasite gel treatment.

These three dressings (intrasite gel, Gelam honey and Nenas honey) provided the wounds with moist healing environment, which promoted the healing process by enhancing epithelialization. Viscosity of honey provided the wound with a suitable environment that enhanced the healing process. These dressings offer the antibacterial barrier to the wound due to its natural characteristic. This study showed the same positive effects on the efficacy of Malaysian honey on wounds inflicted on animals (Aljady, 2000; Khoo et al., 2010). Since the Intrasite gel dressing had the ability to rehydrate the wound and also the necrotic tissue, Intrasite gel dressing showed the same effect as the honey dressing (Ingle, 2006).

A number of clinical trials have reported the effectiveness of topical application of honey in reducing infections in the wounds. There were studies reporting that wounds became sterile on day 3 to day 6 of treatments or day 7 to day 10 of treatments (Cavanagh et al., 1970). The results in this study showed that no infections were found on wounds treated with both type of honey.

The antibacterial activity of honey which was partly due to its high osmolarity could have inhibited the growth of bacteria and also accelerated the cleansing and de-sloughing of dirty wounds (Subrahmanyam et al., 2001). Its viscosity formed a physical barrier which prevented bacterial colonization of wounds (Aljady, 2003).

The main additional antibacterial component in honey is hydrogen peroxide. It provides slow release delivering hydrogen peroxide generated in diluted honey by the action of glucose oxidase enzyme at a steady rate for at least 24 hours. Besides that, the antibacterial property was also contributed by the presence of phenolic acids. This

antibacterial property which contributed to wound healing efficacy was dependent on the floral source of the honey samples (Aljady, 2003).

Normal skin is mechanically and cosmetically superior to the scar tissue. Early contraction in the wound healing process is desirable to produce local deformation (Rozaini et al., 2005). It results in the interaction of the fibroblast locomotion and collagen reorganization in the granulation tissue (Aljady et al, 2000). The results obtained from this study also indicated that honey provided energy needed for the wound contraction.

3.6 CONCLUSION

The efficacy of Gelam honey for wound healing was evaluated again using animal model similar to the previous study done by Aljady (2003). However, different parameters were used in evaluating the wound healing in this study. Nenas honey, another type of Malaysian honey also chosen as a dressing and was evaluated for the first time. The efficacy of these two honey were compared. The effects of both honey were similar but Nenas honey gave lesser scar to the wounds. Thinner scab formed on the wound treated with Nenas honey group; thus, the healing process was faster compared to the Gelam group.

Both honey showed the acceleration of wound healing compared to the untreated groups. Honey provided the wound with a suitable moist environment that enhanced the healing process by stimulating several major events involved in wound healing, such as epithelialization, granulation, and fibroplasias (Aljady et al., 2000 and Rozaini et al., 2005).

Other than that, Intrasite gel treatment also showed similar results in wound healing as the groups treated with honey. In the beginning of the wound healing process, the Intrasite gel treatment was seen to be more effective compared to the honey treatment. It managed to dry up and accelerated the wound contraction. However, this dressing had caused the formation of hard and crusty scab which was attached to the wound. The hard detachment had resulted in the delay of the healing process.

The dressing used in this study showed similar effects in accelerating the wound healing duration to approximately 13 days of treatments. They accelerated the wound contraction, reduced the amount of exudates and necrotic tissue. Besides that, the treatments also provided the moist healing environment to the wounds.

In conclusion, the treatments of both honey showed similar results in accelerating the wound healing process by reducing the size of the wounds and increasing the rate of contractions. The acceleration of the wound healing process was by providing the wound a suitable environment which favored the healing process. It also indicated that honey reduced the formation of scab and necrotic tissue. Nenas honey showed better healing ability in this study. Thus, the macroscopic evaluation of the wound healing showed that Nenas honey gave the best result in wound healing followed by Gelam honey. Intrasite gel gave similar results of healing. However, some side effects such as the formation of hard scab and also the delay in scab detachment had affected the final result. In addition, the cost of Intrasite gel used in this treatment is more expensive than honey.