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The Healing Effect of Honey on First Degree Burns

Ami Febriza[1*], Ahmad Rizal[2]

[1]*Departement of Physiology, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Makassar, Indonesia*
[2]*Students of Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Makassar, Indonesia*

Abstract : *Honey is a safe natural product for treating bacterial colonized wounds. Various previous studies have stated that honey is effective as an alternative treatment for various types of wounds, including burns. However, it is not explained how honey plays a role in the healing process of burns. This research aims to determine the degree of re-epithelialization of first-degree burns facilitated by honey. All mice were induced with first-degree burns. The mice were divided into Group I (control), Group II, (received non-diluted pure honey), Group III (treatment of diluted honey (10 ml honey in 10 ml distilled water), and Group IV (treatment of diluted honey (10 ml honey in 20 ml distilled water). The intervention was conducted for 7 days. Wound size and wound healing time were analyzed by ANOVA. Based on the results, it was concluded that there were at least differences in the durations of the burn wound healing process and differences in the size of the burn area between the four groups during one week of observation (p-value <0.000). Compared to the control group that was not treated with honey, there was a clinical effect of topical application of pure honey on burn healing in the treatment groups.*

Keywords – *honey, wound healing, burns*

I. INTRODUCTION

Honey is one of food substances made by bees that has high nutritional value. Honey is a substance produced by bees from sugary secretions of plants, or floral nectar, that are then changed and combined with special substances in bees and stored to ripen in honeycombs. There is special substance in bees that function in the process of breaking down sugar, namely, hydrolase enzyme in the watery saliva of bees. This enzyme is added by worker bees when they drink and regurgitate nectar and serves to convert sucrose into dextrose (glucose) and levulose (fructose) [1]. Honey has long been used as medicine, and research conducted in the last decade has shown the immense benefits of honey. In wound care, honey is a natural substance that is safe for treating wound that is colonized or infected by bacteria [2]. In addition to having an anti-microbial effect, honey also has an anti-inflammatory effect and increases fibroblastic and angioblastic activity [3].

Superficial first-degree burns involve only the epidermis. Healing occurs spontaneously in 10-14 days without scarring. Wound healing is about the quality of tissue life. It is also associated with tissue regeneration. The healing process can occur normally without treatment, although some treatments can help to support the healing process [4] Burn healing goes through several phases, namely, the inflammatory phase, the proliferative phase, and the maturation phase. The process of epithelialization occurs during the proliferative phase. Layers of cells that die from trauma protect living cells in the deeper layers of the epithelium. Granulation tissue is formed

by the integration of collagen synthesized by fibroblasts with ground substance. During wound healing, the cells at the wound edges develop into a thin layer that spreads over the gaps in the epithelium. On the other hand, at the wound edges, cell division begins somewhat later to provide the necessary cells for the recovery of the epithelium until it reaches its normal thickness [5], [6].

1.1. Nutritional Composition of Honey

Natural honey contains about 200 substances, including amino acids, vitamins, minerals and enzymes, but mainly contains sugar and water. Sugar is almost 95-99% of the constituent substances of dry honey. The main types of carbohydrates in honey are fructose (32.56% to 38.2%) and glucose (28.54% to 31.3%), which constitute 85-95% of the total sugar present and are easily absorbed in the digestive tract [7]–[9].

Other sugars contained in honey include disaccharides such as maltose, sucrose, isomaltose turanose, nigerose, meliose, panose, maltotriose, and melezitose. Honey also consists of several oligosaccharides. In addition, it contains 4-5% fructooligosaccharides, which function as probiotic substances [7][10]. Water is also the most important component. Organic acids, including gluconic acid that is a by-product of the enzymatic digestion of glucose, represent 0.57% of honey's constituent. Organic acids are responsible for the acidity of honey and contribute greatly to its distinctive taste [11]. The concentration of mineral compounds in honey is about 0.1-1.0%. The main mineral is potassium. Other minerals such as calcium, magnesium, sodium, sulfur, phosphorus, iron, zinc, and manganese are also found in honey [12], [13]. The nutritional composition of honey is presented in Table 1[13].

Table 1. Nutritional Composition of Honey [13]

Nutritional Composition in 100 mg Honey	
Carbohydrate	82.4 g
Fructose	38.5 g
Glucose	31 g
Sucrose	1 g
Other types of sugar	11.7 g
Fiber	0.2 g
Fat	0 g
Protein	0.3 g
Water	17.1 g
Riboflavin (Vitamin B_2)	0.038 mg
Niacin (Vitamin B_3)	0.121 mg
Panthothenic Acid (Vitamin B_5)	0.068 mg
Pyridoxine (Vitamin B_6)	0.024 mg
Folic acid (Vitamin B_9)	0.002 mg
Vitamin C	0.5 mg
Calcium	6 mg
Iron	0.42 mg
Magnesium	2 mg
Phosphate	4 mg
Sodium	52 mg
Potassium	4 mg
Zinc	0.22 mg

1.2. Therapeutic Activities of Honey in Wound Healing

The molecular pathomechanism in the wound healing process facilitated by honey has not been elucidated. However, the result of a study recommends that the use of appropriate wound dressings with honey therapy is sufficient to provide satisfactory results. The type of wound and the severity of the wound affect the efficacy of honey in healing wounds. The honey chosen must be used in sufficient quantities so that its concentration will remain adequate even if it is diluted with wound exudate. The therapeutic result is better if honey is applied to a dressing than if it is applied to the wound. All wound cavities should be smeared with honey and filled with dressings to prevent exudate from being produced [14], [15]. In burns, honey has a cooling or soothing effect on initial administration, which is then followed by rapid wound healing. Honey has been used as a wound dressing for tumor implantation in laparoscopy. No infections have been reported from administering honey to open wounds. Honey also plays a potential therapeutic role in the treatment of gingivitis and periodontal disease [15]. Similar results were found with burns. Dressings with topical application of honey expedite the wound healing process, sterilize the wound, and reduce pain [16]. According to a study of cases of gangrene, the use of honey as a therapeutic substance resulted in a reduction in edema and the amount of exudate, rapid epithelial regeneration and little or no scar tissue formation, effective wound debridement, and reduced mortality [17].

Honey is used to treat ulceration after radical surgery in breast cancer and varicose veins. In addition, honey is used after radical surgery for vulvar cancer to provide infection-free postoperative wound results with minimal wound debridement and to shorten hospital stay [18]. In patients with postoperative wound infections after cesarean section or hysterectomy, topical application of honey causes a more rapid reduction in the number of infecting bacteria, reduces antibiotic use and duration of hospital stay, expedites wound healing, and results in minimal scar tissue formation [19]. Clinical trials were conducted by comparing dressing with topical application of honey on burns with amniotic membrane-assisted dressing; sulfadiazine cream with boiled potato peel dressing. Dressing with topical application of honey resulted in faster wound repair and early healing with lower rates of contractures and scar tissue formation [14], [20]. Research by Motallebnejad et. Al. (2008) reported that topical application of honey was effective in the treatment of radiation-induced mucositis [21].

1.3. Wound Healing

Wound healing is a complex process that results in restoration of tissue integrity. This process is divided into four phases, namely hemostasis, inflammation, proliferation, and tissue remodeling [22], [23]. The hemostasis phase is a process that occurs immediately as an initial response to injury. This response involves the role of platelets and the coagulation cascade to prevent extravasation and establish hemostasis. The inflammatory phase in wound healing aims to prevent infection. This process is traversed through various chemical signaling mechanisms including the complement cascade, interleukin activation and signaling transforming growth factor beta (TGF-β) resulting in chemotaxis [24], [25]. Cells that play a role in this phase such as neutrophils, macrophages, lymphocytes, fibroblasts, and endothelial cells. The inflammatory phase of wound healing will last as long as bacteria and debris remain in the wound. The proliferative phase begins when the wound stimulus has stopped, hemostasis has occurred, the inflammatory response has stabilized and there is no more debris, consisting of the process of angiogenesis, granulation tissue formation, collagen deposition, epithelialization and wound retraction at the same time. The final phase of wound healing process is the remodeling phase that causes normal epithelial development and maturation of scar tissue [24].

Many previous studies have stated that honey is effective as an alternative treatment for various kinds of wounds, including burns. However, it has never been explained how honey plays a role in the healing process of burns. Based on this, this research aims to determine the degree of re-epithelialization of first-degree burns facilitated by honey.

II. MATERIAL AND METHODS

2.1. Preparation and Adaptation of Animal for Research

This stage was the stage of preparation and adaptation of experimental animals. The mice used were male *Mus musculus* var. albino, who were aged 12-16 weeks and weighed 25-35 gr. Mice were placed in cages

made of wire with a floor area of 30 cm x 50 cm x 15 cm. Each cage contained 7 mice. Each mouse was fed 300 g/day and provided with sufficient water to drink, and the cage was cleaned every day. To maintain a stable environmental atmosphere, mice were placed in a room with sufficient air circulation, temperature that was maintained in accordance with the room temperature at standard conditions (28 ± 2°C), and humidity that was 50%±10%, and the room light was turned on for 12 hours and for the next 12 hours, was turned off. This cycle and other procedures were done every day for a week. Next, randomization was conducted. All mice were then grouped into 4 groups randomly. Group I was the control group (no treatment), Group II was the group that received non-diluted natural honey treatment, Group III was the group that received diluted honey treatment (10 ml of honey in 10 ml of distilled water), and Group IV was the group that received diluted honey treatment (10 ml of honey in 20 ml of distilled water).

2.2. Induction of First-Degree Burns in Mice

The backs of the mice were shaved, then disinfected using povidone-iodine or alcohol 70%. Then, anesthesia was performed around the area to be burned. After that, the samples were made to have first-degree burns using ±1 cm (hot) plate, which was modified and heated with a soldering iron (gun model 30-100W/AC 220V-240V), with the solder lever not pressed (providing 30W of heating), then placed on the skin of mice for 1 second.

2.3. Topical Application of Honey

The honey used was natural honey that has passed a test, which was then diluted with distilled water and grouped into three: 1) 10 ml undiluted natural honey, 2) 10 ml natural honey diluted with 10 ml distilled water, 3) 10 ml natural honey ml diluted with 20 ml of distilled water. Honey was diluted in volumetric flasks and the results were applied to mice that had been treated with first-degree burns and met the inclusion criteria as sample. Honey was applied using a cotton bud. Burns were smeared with honey and covered with sterile gauze. Honey was applied twice a day, in the morning and evening. Observations and treatments were conducted for 7 days.

2.4. Statistical Analysis

The Shapiro-Wilk normality test was conducted on the research results to determine the normality of the data distribution. Then, Levene's test was conducted to determine the data variance. Data that were normally distributed and homogeneous were tested using a parametric test, one-way ANOVA. Meanwhile, non-normally distributed data were tested by Kruskal-Wallis non-parametric test. The test results which showed significant data ($p<0.05$) were followed up with Post Hoc analysis to see the differences between the intervention groups.

III. RESULTS

The indicators assessed in the wound were hyperemia and edema in first-degree burns. It is said to be successful or fully healed if there are no hyperemia and edema. First degree burns in samples that was gradually healing were measured and the length of healing time (in days) and the size of the wound area (cm) in one-week observation in each sample were compared. Figure 1 shows the average healing time for burns in each group.

3.1. Data Characteristics

The sample consisted of four groups, each of which consisted of seven mice. Data regarding the length of time for healing (in days) and wound area (in cm) after treatments are shown in Table 1. Based on the results of statistical tests, it is concluded that at least, there were differences in the length of time for wound healing and differences in wound size during one week of observation of four group.

Table (1): Data Characteristics

Variables	Group I (n=7)	Group II (n=7)	Group III (n=7)	Group IV (n=7)	*p-value*
Wound healing (in days)	6.0±0.6	3.9±0.3	4.9±0.2	6.1±0.5	0.000
Wound size (cm)	0.46±0.04	0.24±0.03	0.36±0.04	0.45±0.05	0.000

Data in Table.1 are presented in "mean±standard deviation". Data on wound size were normally distributed, while data on wound healing time were not normally distributed. The p-value <0.05 was used for comparison between groups (Kruskal-Wallis test for data that were not normally distributed and one-way ANOVA for data that were normally distributed).

The real difference can be seen in Figure 1. The group of mice with burns that were treated with pure honey topically showed faster wound healing. The groups of mice treated with honey that had been diluted with distilled water exhibited slower wound healing.

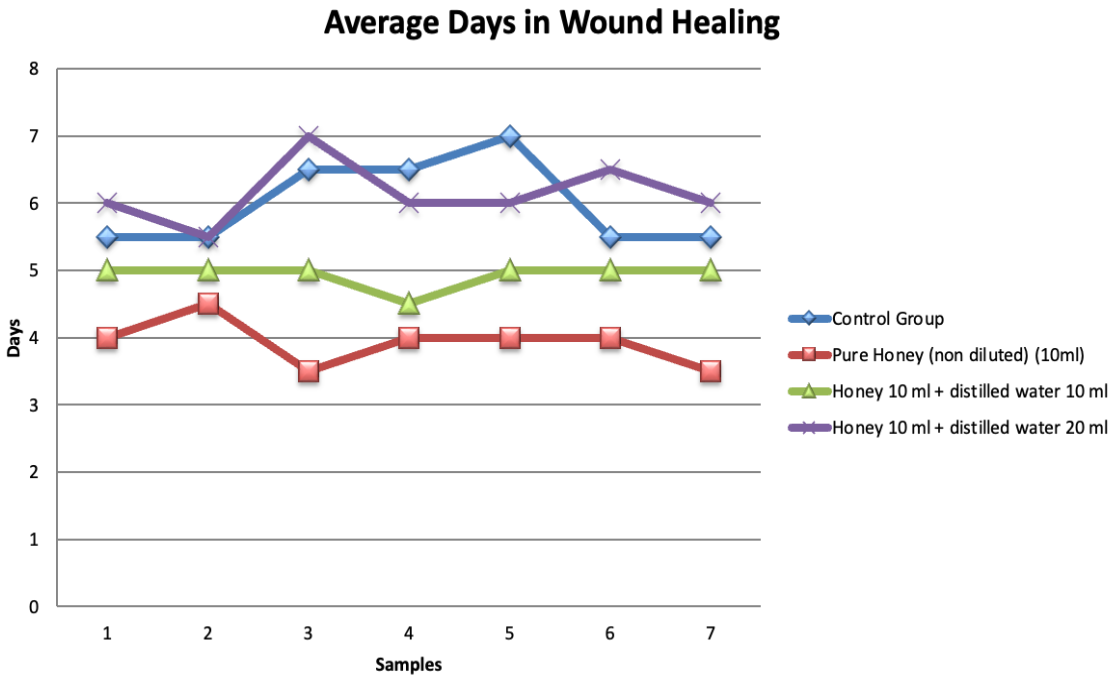

Fig (1): Average Wound Healing Time (in Days)

The observations presented in Figure 2 indicate that each group of mice had a different healing pattern based on the size of the wound observed for one week.

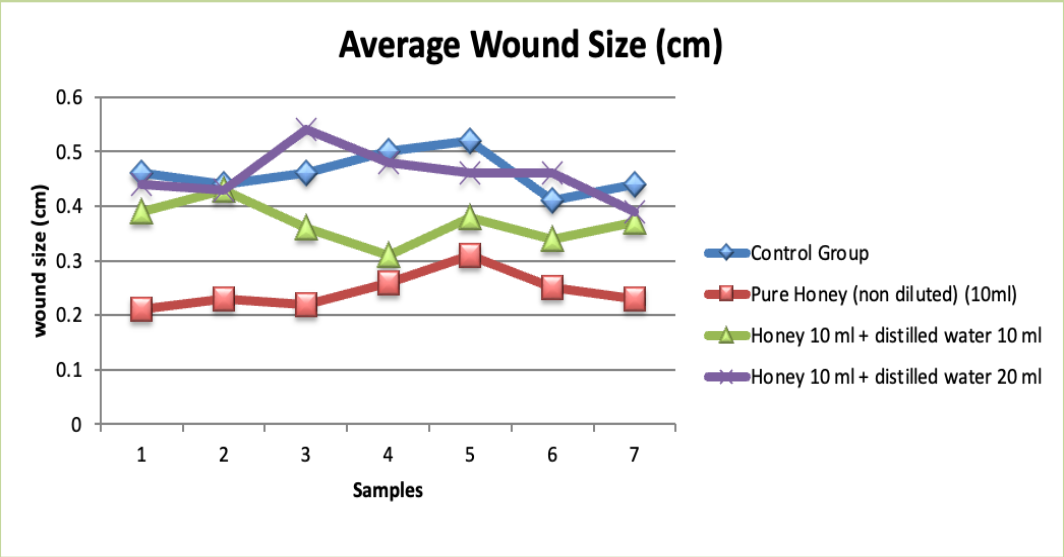

Fig (2): Average Wound Size during One Week of Intervention

The results of direct observations of two groups, namely Group I (control group) and Group II (natural honey treatment group), can be seen in Figure 3 and Figure 4.

(A) (B)

Fig (3): Day-1. In Group I (Control Group) (A), hyperemia and edema were observable in the burns and the burn area was still extensive. In Group II, with the topical administration of undiluted natural honey (B), hyperemia and subtle edema were seen in the burns, and the burn area was reduced.

(A) (B)

Fig (4): Day-4. In Group I (Control Group) (A), hyperemia was still observable in the burn site, the burn area was reduced, and edema was not detected. In Group II, with the topical administration of undiluted natural honey (B), the skin color was observable at the burn site, the burn area was not detected, and there was no edema.

IV. DISCUSSIONS

In the results presented, all groups observed for one-week experienced complete wound healing. On day 1, pure honey was applied on Group I and the pure honey was able to reduce the effect of burns and the expansion of burns, while at the same time, the control group did not experience any changes. Honey contains active substances that are able to influence local cellular and molecular activity and also act as a stimulus for cells in the wound healing process, such as through the expression of proinflammatory cytokine genes, during the inflammatory stage of healing. On the other hand, the wound healing process in the control group was slow because the control group was not provided with drugs or substances that can expedite wound healing. However, the control group still healed because the cells regenerate naturally but slowly. This is because the production of proinflammatory cytokines is reduced in the early stages of wound healing. Healing is also influenced by a healthy body having a natural ability to protect itself and recover.

The time required for the group facilitated by natural honey to heal from wounds was shorter than the healing time for the control group. This was influenced by the active substances contained in natural honey, namely, flavonoids and other honey compositions. Flavonoids act as antioxidant and antibacterial agents. Flavonoids are one of the most common groups of secondary metabolites found in plant tissues. These compounds act as antioxidants by donating hydrogen atoms or by their ability to chelate metals, being in the form of glucosides. Flavonoids have a composition of 90% diosmin and 10% hesperidin, which have the effect of increasing vascularity and protecting the vascular endothelium. Based on the results of clinical studies and experiments, flavonoids can increase vascularization and reduce edema. Meanwhile, the antibacterial effects derive from flavonoids, namely apigenin, galangin, pinocembrin, ponciretin, genkwanin, sophoraflavanone G and its derivatives, naringin, naringenin, epigallocatechin gallate and its derivatives, luteolin, luteolin 7-glucoside, quercetin, 3-O-methylquercetin, quercetin glycosides, kaempferol and their derivatives. Other types of flavonoids are flavone glycosides, isoflavones, flavanones, isoflavanones, isoflavans, flavonols, flavonol glycosides, and chalcones. Flavonoids can damage cells by inhibiting macromolecular synthesis[11].

In Group 3 (treated with 10 ml natural honey + 10 ml distilled water), the average healing time was 5 days, while in Group 4 (treated with 10 ml natural honey + 20 ml distilled water), the average healing time was 6-7 days. The reduced effectiveness or effect of honey in Group 3 and Group 4 is suspected to be due to dilution with distilled water that causes the consistency of honey to change, in terms of viscosity, osmolarity, and pH, which then causes the distribution of honey composition to be inadequate so that the flavonoid content is also

inadequate because of the dilution effect by distilled water. Therefore, the rate of burn healing was faster in burns facilitated by natural honey without dilution with an adequate composition [26].

The inflammatory phase starts right after injury and lasts 2-3 days, starting with vasoconstriction to achieve hemostasis. A thrombus is formed and blood coagulation is activated, resulting in fibrin deposition. Platelets release platelet-derived growth factor (PDGF) and transforming growth factor (TGF-β) that attract inflammatory cells, especially macrophages. After hemostasis is achieved, vasodilation occurs and vascular permeability increases, the neutrophil count peaks at 24 hours and neutrophils drive debridement, and monocytes enter the wound, become macrophages, and their number peaks in 2-3 days. Macrophages produce PDGF and TGF-β that will attract fibroblasts and stimulate collagen formation. It is continued by the proliferative phase, which started on day 3. Fibroblasts are attracted and activated by PDGF and TGF-β, enter the wound on day 3, reaching the highest number on day 7. Synthesis of collagen (especially type III collagen), angiogenesis, and epithelialization occur. The amount of collagen increases until the production and degradation of collagen reach equilibrium, which marks the start of the remodeling phase [23]. Undiluted pure honey contains active substances that expedite the inflammatory and proliferative process in mice treated with undiluted pure honey. Honey is hygroscopic, which means it absorbs water so that it becomes dilute and will absorb moisture, thereby reducing the bad effects of damaged cells due to hot objects so that the edema in the samples treated with pure honey healed faster than the edema in the samples in the control group. Honey contains flavonoids which act as antimicrobial and antioxidant agents. Flavonoids in this case shorten the inflammatory process because there are no foreign objects (bacteria) during the inflammatory process [26].

V. CONCLUSION

Compared to the control group that was not treated with honey, there was a clinical effect of topical application of pure honey on burn healing in the treatment groups, but the differences in the effects between the control group and the treatment groups were not significant. With a rich content of nutrients and flavonoids as antioxidant and antibacterial agents, it is possible that in this experiment, honey, especially pure honey, affects the healing of first-degree burns by expediting the rate of burn healing.

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REFERENCES

[1] J. M. Alvarez-Suarez, S. Tulipani, S. Romandini, E. Bertoli, and M. Battino, Contribution of honey in nutrition and human health: a review, *Med. J. Nutrition Metab., 3(1),* 2010, 15–23.

[2] C. Dunford, R. Cooper, P. Molan, and R. White, "The use of honey in wound management.," *Nurs. Stand., 15(11),* 2000, 63–68.

[3] B. Medhi, A. Puri, S. Upadhyay, and L. Kaman, Topical Application of Honey in The Treatment of Wound Healing : A Metaanalysis, *JK Science, 10(4),* 2008.

[4] E. C. O. Lloyd, B. C. Rodgers, M. Michener, and M. S. Williams, Outpatient Burns: Prevention and Care, *Am Fam Physician, 85(1),* 2012, 25–32.

[5] H. Sorg, D. J. Tilkorn, U. Mirastschijski, and S. Hager, Skin Wound Healing : An Update on the Current Knowledge and Concepts, *Eur Surg Res, 58(1-2),* 2017, 81–94.

[6] F. M. Thiruvoth, D. P. Mohapatra, D. K. Sivakumar, R. K. Chittoria, and V. Nandhagopal, Current concepts in the physiology of adult wound healing, *Plastic and Aesthetic Research, 2(5),* 2015, 250-256.

[7] A. M. Ezz El-Arab, S. M. Girgis, E. M. Hegazy, and A. B. Abd El-Khalek, Effect of dietary honey on intestinal microflora and toxicity of mycotoxins in mice.,*BMC Complement. Altern. Med.*, vol. 6, p. 6, Mar. 2006, doi: 10.1186/1472-6882-6-6.

[8] P. Missio, C. Gauche, L. V. Gonzaga, C. O. Costa, and R. Fett, "Honey: Chemical composition, stability and authenticity," *FOOD Chem.*, 2015, doi: 10.1016/j.foodchem.2015.09.051.

[9] D. W. Ball, The Chemical Composition of Honey, *J. Chem.Educ*., *84(1)*, 2007, 1643–1646.

[10] J. Chow, Probiotics and prebiotics: A brief overview., *J. Ren. Nutr., 12(2)*, 2002, 76–86.

[11] P. B. Olaitan and O. E. Adeleke, Honey : a reservoir for microorganisms and an inhibitory, *Afr.Health Sci, 7(3)*, 2007, 159–165.

[12] J. Lachman, D. Kolihová, D. Miholová, J. Košata, D. Titěra, and K. Kult, Analysis of minority honey components: Possible use for the evaluation of honey quality, *Food Chem.*,101(3), 2007, 973–979.

[13] M. N. Rashed and M. E. Soltan, Major and trace elements in different types of Egyptian mono-floral and non-floral bee honeys, *J. Food Compos. Anal., 17(6)*, 2004, 725–735.

[14] V. Bansal, B. Medhi, and P. Pandhi, Honey--a remedy rediscovered and its therapeutic utility., *Kathmandu Univ. Med. J. (KUMJ)*, *3(3)*, 305–309, 2005.

[15] P C. Molan, The evidence and the rationale for the use of honey as a wound dressing, *Int J Low Extrem Wounds, 19(4)*, 2011, 40-54.

[16] M. Subrahmanyam, Topical application of honey in treatment of burns., *Br. J. Surg., 78(4)*, 1991, 497–498.

[17] M. Gürdal, E. Yücebas, A. Tekin, M. Beysel, and R. Aslan, Predisposing Factors and Treatment Outcome in Fournier's Gangrene,*Urol. Int. - UROL INT*, *70*, 2003, pp. 286–290.

[18] D. Cavanagh, J. Beazley, and F. Ostapowicz, Radical Operation For Carcinoma Of The Vulva, *BJOG An Int. J. Obstet. Gynaecol.*, 77(11), 1970, 1037–1040.

[19] N. S. Al-Waili, Mixture of honey, beeswax and olive oil inhibits growth of Staphylococcus aureus and Candida albicans., *Arch. Med. Res., 36(1)*, 2005,10–13.

[20] A. Meda, C. E. Lamien, J. Millogo, M. Romito, and O. G. Nacoulma, Therapeutic uses of honey and honeybee larvae in central Burkina Faso.,*J. Ethnopharmacol., 95(1)*, 2004, 103–107.

[21] M. Motallebnejad, S. Akram, A. Moghadamnia, Z. Moulana, and S. Omidi, The effect of topical application of pure honey on radiation-induced mucositis: a randomized clinical trial., *J. Contemp. Dent. Pract., 9(3)*, 2008, 40–47.

[22] M. E. Okur, I. D. Karantas, Z. Şenyiğit, N. Üstündağ Okur, and P. I. Siafaka, Recent trends on wound management: New therapeutic choices based on polymeric carriers., *Asian J. Pharm. Sci., 15(6)*, 2020, 661–684.

[23] H. N. Wilkinson and M. J. Hardman, Wound healing: cellular mechanisms and pathological outcomes.,*Open Biol., 10(9)*, 2020, 200-223.

[24] C. Tsioufis, I. Bafakis, A. Kasiakogias, and C. Stefanadis, The role of matrix metalloproteinases in diabetes mellitus., *Curr. Top. Med. Chem., 12(1)*, 2012, 1159–1165.

[25] B. Berman, A. Maderal, and B. Raphael, Keloids and Hypertrophic Scars: Pathophysiology, Classification, and Treatment.," *Dermatologic Surg. Off. Publ. Am. Soc. Dermatologic Surg. [et al.]*, *43(1)*, 2017, 3–18.

[26] A. Redha, Flavonoid: Struktur, Sifat Antioksidatif dan Peranannya Dalam Sistem Biologis, *J. Berlin, 9(2)*, 2010, pp. 196–202.