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Effectiveness of Honey and Moist Exposed Burn Ointment (MEBO) in Epithelial-Burns of Grade II

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Abstract : Second degree burns treatment plays an important role in healing burns. Many people use Moist Exposed Burn Ointment (MEBO), an oil-based ointment that has been proposed for the treatment of ideal burns, and honey has also been used for the treatment of burns in several clinical trials and provides good results. This study aims to determine the ratio of honey and MEBO effectiveness in epithelialization of second degree burns.Methodsby using the true experiment research method or pure experiment with RAL (Complete Random Design). Results The results showed that giving honey to healing burns gave the best results where there were significant differences ($p < 0.05$) in the formation of collagen fibers and the amount of blood vessel formation compared to MEBO, but in the results of measurements of the extent of burns and epithelial formation did not showed a significant difference ($p > 0.05$) between honey and MEBO.Conclusion/ConclusionsThe conclusion is that giving honey gives better and more effective results in the formation of collagen fibers and blood vessel formation compared to MEBO. Therefore honey can be an option for the community in dealing with second degree burns, and further research needs to be done on signal transduction (molecular biology) related to wound healing in three phases, namely the inflammatory phase (eg interleukin 2, 6 and others). others), proliferation phase (eg EGF, PDGF, TGF-ß) and remodeling phase (eg BMP2).
Keywords : burns, honey, MEBO, epithelialization, collagen fibers, blood vessels.

Introduction

Burns are a form of damage and / or tissue loss caused by contact with sources that have very high temperatures (eg fire, hot water, chemicals, electricity and radiation) or very low temperatures[1], it could also be due to exposure to **high** temperatures from the sun and burns due to scalded water that often occur in household accidents[2]. Burns are injuries that are quite often faced by doctors and in handling them require a high cost. Burns care plays an important role in healing burns[2]. Globally around 11 million people with burns will seek medical treatment, there are 300,000 deaths from burns every year[3]

About 90% of burns occur in developing countries[3].Overall, almost 60% of fatal burns occur in Southeast Asia, with an incidence rate of 11.6 per 100,000 inhabitants[4].Every year around 2.5 million cases of burns occur in Indonesia, more than 250 people per year die from burns[1]. An estimated 500,000 burn injuries require medical treatment each year in the United States, with around 40,000 hospitalizations and 3400 deaths[5].

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Life expectancy has increased consistently in the past 4 decades, because now almost 97% of patients are treated at the burn center[5]. For burns, many people use ointments from natural ingredients, namely Moist Exposed Burn Ointment (MEBO), oil-based ointments containing sesame oil, beta-sitosterol, beberine, and a small amount of other plant materials developed at the Chinese National Science and Technology Center in Beijing, China in 1989 which has been proposed as an ideal treatment for burns[6].

MEBO began to be popular with its use in the treatment of burns because the healing process was relatively fast[7]. MEBO is believed to protect wounds from infection and accelerate wound healing without side effects[8]. But MEBO has a relatively high price compared to other types of burn medicine[7].

Other natural ingredients that are famous for their efficacy in the field of health and beauty are honey. According to Jose and Massimiliano's research, honey is included in natural ingredients that are beneficial as antioxidants and anti-bacterial but do not cause a risk of resistance[9].

Honey has high osmolarity, and also has antibacterial properties, namely hydrogen perioxide. Other honey content is composed of 17.1% water, 82.4% total carbohydrates and 0.5% protein, amino acids, vitamins and minerals. With this content honey has the ability to clean wounds, absorb edema fluid, trigger tissue granulation, epithelialization and increase nutrition. Apart from the price of honey itself, it is still quite cheap compared to the standard medicine for burns. But the use of honey is still not widely used in the professional sphere[10].

Methods

Sample

Male adult white rats obtained from the Faculty of Biology, Universitas Sumatera Utara. The rats sampled weighed around 150-250 grams and numbered 20 for 4 treatment groups (control, honey, MEBO) with each group consisting of 5 rats namely. (A) Group 1 (positive control): sterile burns + sterile aquades, B. Group 2 (honey): burns + topical honey 2x a day on days 3,7,14, 21, (C). Group 3 (MEBO): topical burn + mebo 2x a day on day 3,7,14,21, dan D. Group 4 (negative control): no treatment

Procedure

Before the treatment of all laboratory rats, 20 white rats were adapted at the Biology Laboratory of the Faculty of Medicine, University of North Sumatra for 2 weeks. After the adaptation period, the rats are separated into one cage containing one mouse. After that, burns were given to the skin of the rats by means of mouse hair shaved in the area to be given a burn and the cloth was placed below as a base for giving burns. Then local anesthesia was given to the skin with a dose of 0.2 cc lidocaine in 2 cc of aquadest. Next, heated the iron plate and put it on the back skin of the rats that had been prepared with the same area until bullae formed. After the burns were formed, honey and MEBO were applied, while in the positive control group the treatment of burns only used aquadest and negative control groups that were given no treatment.

Procedure for Treatment of Degree-II Burns

Handling of burns must be careful so cleaning must be done first using aquadest before giving MEBO or honey ointment. On applying the medication, burns are done immediately after a burn is given twice a day. The steps for handling burns are prepared gauze and arranged the position of rats to facilitate action. Smeared the wound with gauze that has been moistened with 2-3 mm thick honey to cover the entire surface of the wound. As for the treatment group with MEBO, the wound section was smeared using 2-3 mm thick MEBO to cover the entire wound surface. Then the wound is closed with sterile gauze. In the control treatment group, the wound was covered with 3 layers of sterile gauze moistened with distilled water, then placed on a second degree burn, then covered with 5 sterile gauze layers. The dressing is replaced if the condition is saturated. Handling of burns with honey, MEBO, and sterile aquades control group was carried out for ± 21 days. Observations were carried out on days 4,7, 14 and 21.

The process of observing wound contractions is carried out according to the procedure[10] with modifications

Observation of the wound using a SUSMANT Wound Healing Tool observation sheet to observe hemorrhage, maceration, undermaining, erythema, necrosis, wound edges, granulation, contraction, continuous contraction and epithelialization. I performed on day 3,7,14 and 21.

Histology analysis of healing burns

Microscopic assessment of wound healing was seen at 40x magnification in 3 fields of view in each specimen using the results of anatomical pathology examination of wound incisions biopsy which included; (1) Level of collagen formation, (2) Level of epithelialization, and (3) The number of new blood vessel formation and number of inflammatory cells with criteria modified[11,12].

Making preparations namely control rats and treatment in anesthesia using ether-pressed cotton in a jar. Rats were put into jars and awaited until anesthetized rats. Then the skin preparation measuring 1.5 x 1.5 cm is taken. Skin samples were taken on days 3,7,14 and 21. Cut skin was fixed with 10% BNF (Buffer Neutral Formaline) solution. Then the sample was sent to the Histopathology Laboratory of the Faculty of Medicine, University of North Sumatra, for the preparation of preparations for skin preparations that had been fixed using Neutral Formalin Buffer or 10% BNF then trimming, dehydrated with multilevel alcohol, then embedding or further paraffinizing the frozen paraffin cut with microtome 5 μm thickness and then do the coloring process with HE and Mallory. The process of measuring the thickness of collagen and epithelialization was carried out by taking skin tissue preparations to make histological slides with vertical cutting using HE staining (Hematoxilin-Eosin) for epithelialization while Mallory was used for observation of collagen and new blood vessel formation or angiogenesis. Histology slides were then scanned using Olyvia software and carried out 40x and 4x magnifications. The wound section was screen printed and entered into the AutoCAD 2009 software. Measurement of epithelial thickness and thickness of collagen based on research conducted by granulation tissue was measured from the base of the wound down to the lower dermis where fibroblast cell proliferation ended[13]. Measurements are made in three different areas, namely on the left, mid and right sides. After getting the results from the measurement of the three areas, the average value is taken. Ethical clearance for Animal Research Ethical Cleancre Universitas Sumatera Utara number 0096/KEPH-FMIPA/2019

Results and Discussion

Result

Based on the results of research conducted at the Biology Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara Anatomical Pathology Laboratory, Faculty of Medicine, University of North Sumatra, the results are as follows.

Area of Burns

To see the difference in significant differences in the process of wound contraction macroscopically between MEBO and honey in second degree burns, measurements were made of the area of burns formed as shown below. In Figure 1, the healing of burns in rats occurred on the 21st day. Healing was shown by decreasing the wound area both in the treatment of honey and MEBO ($p<0.05$). The use of MEBO and honey was not significantly different in wound healing seen from the side of the reduction in the extent of burns ($p>0.05$). This is because MEBO and honey have their respective advantages in encouraging wound healing.

Table 1. The results of the analysis of the average epithelial thickness of burns in each treatment for 3 days, 7 days, 14 days and 21 days.

Observation (days)	Treatment			
	K+	Honey	MEBO	K-
H3	248,33±8,33^{a}	337,00±15,39^{b}	318,33±15,31^{b}	222,67±11,02^{a}
H7	513,33±13,65^{c}	743,67±16,44^{f}	719,33±17,56^{f}	483,33±9,87^{c}
H14	622,00±10,58^{e}	858,67±20,50^{g}	824,33±15,04^{g}	560,00±60,83^{d}
H21	635,67±12,50^{e}	869,33±26,35^{g}	855,33±15,70^{g}	577,00±58,03^{d}

Note: $p^{a,b}<0.05$ between treatment groups and observations (days)

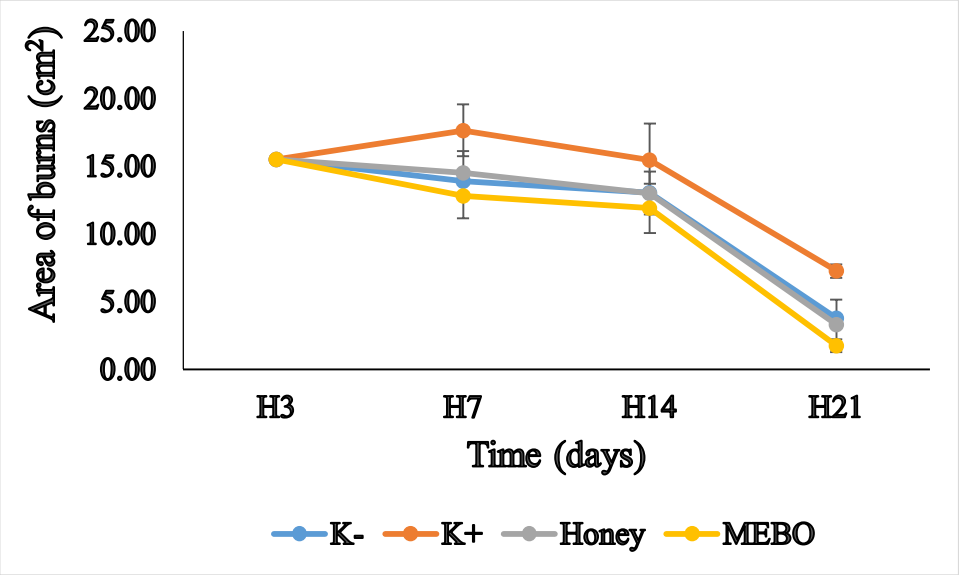

Figure 1. Area of burns (cm^2) of rats after several days of observation and treatment in the study. * p <0.05.

Epithelial thickness

Analysis of significant differences in epithelialization between MEBO and honey in second degree burns was done by measuring burn epithelial thickness as shown in Figure 2.

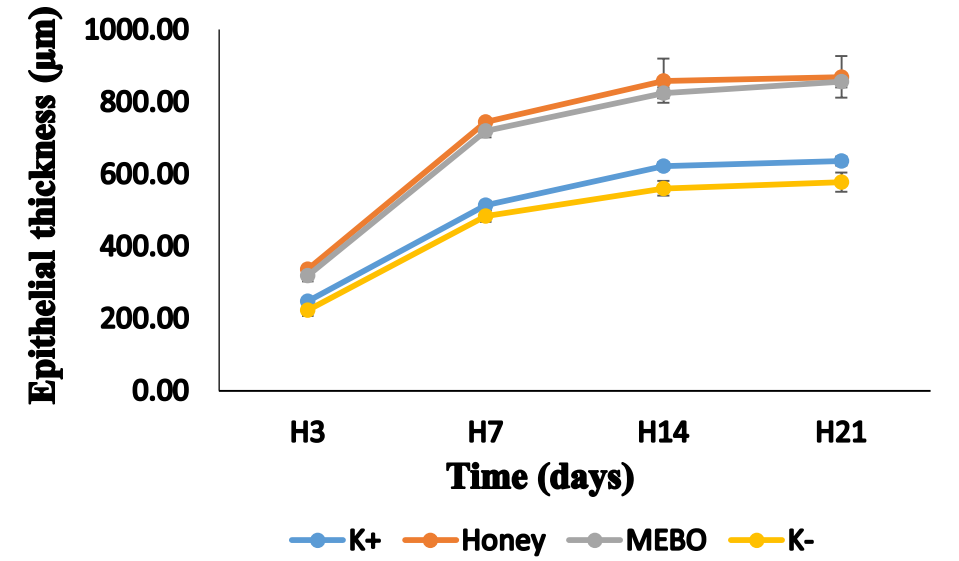

Figure 2. Average epithelial thickness of burns (cm^2) of rats after several days of observation and treatment in the study.

In Figure 2 and Table 1 it can be seen that the epithelial process or formation of epithelial cells that support the occurrence of wound healing is more evident on day 21st. Epithelial thickening occurs due to the process of forming epithelial cells resulting from cell proliferation (Figure 5). The cells formed migrate towards the epithelium and eventually cover the burn. Epithelial thickening occurred in honey and MEBO treatments which were not significantly different between the two ($p>0.05$). But when compared with positive and negative controls, honey and MEBO treatment was very significant ($p<0.05$). As research[17] shows, the administration of honey can increase the thickening of the skin epithelium of rats with burns.

Formation of collagen fibers

The difference in collagen formation between administration of honey and MEBO on healing second degree burns based on the analysis is seen in Table 2 and Figure 3. Density of collagen fibers in very tightly wound areas was seen in the treatment of giving honey and MEBO to second-degree burn rats. Between Honey and MEBO there was no significant difference in the formation of collagen fibers. This is due to the content of honey and MEBO which strongly supports the formation of collagen fibers in rat burns, such as vitamin C (ascorbic acid)[19]stated that vitamin C can trigger the formation of collagen. Honey contains vitamin C (ascorbic acid) because most plant flowers that bees like contain these vitamins which function as antioxidants in addition to many other functions. It has been proven that the antioxidant activity of honey is highly dependent on the origin of the plants it inhabits. Associated with the vitamin C content, that the content of vitamin C has a significant impact on the total antioxidant activity of honey[20].

Table 2. Results of analysis of collagen formation in healing burns in each treatment for 3, 7, 14 and 21 days.

Observation (days)	Treatment			
	K+	Honey	Mebo	K-
H3	0.67 ± 0.58^{abc}	1.00 ± 0.00^{abc}	1.00 ± 0.00^{ac}	0.67 ± 0.58^{ac}
H7	1.33 ± 0.58^{abc}	2.00 ± 0.00^{ab}	1.67 ± 0.58^{ac}	1.00 ± 0.00^{c}
H14	2.00 ± 0.00^{ac}	3.00 ± 0.00^{bc}	2.33 ± 0.58^{ac}	2.00 ± 0.00^{a}
H21	3.00 ± 0.00^{abc}	4.00 ± 0.00^{b}	3.33 ± 0.58^{ac}	2.67 ± 0.58^{ac}

Description: $p^{a,b}<0.05$ in the Mann Whitney test between treatment and observation groups.

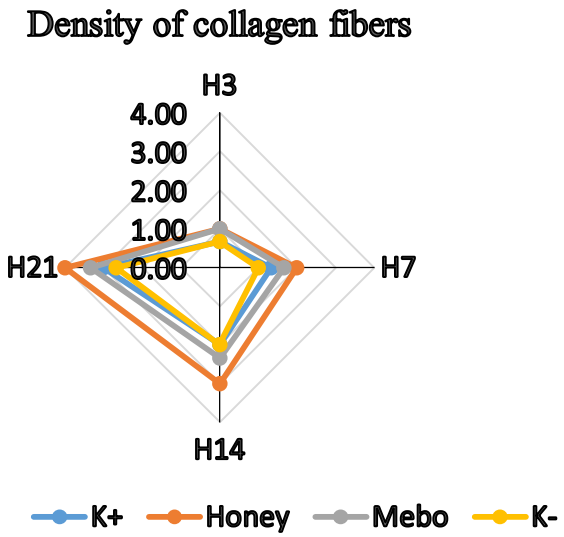

Figure 3. Average density of collagen fibers in the wound area after several days of observation and treatment in the study. Description: 0: no collagen fibers found in the wound area, 1: density of collagen fibers in the low wound area, 2: density of collagen fibers in the area of moderate injury, 3: density of collagen fibers in the area of tight wound, 4: density of collagen fibers in the area very tight wound.

Number of veins

A significant difference in the amount of vascularization (vascular) formation between honey and MEBO in the healing of second degree burns is based on analysts as follows (Figure 4).The number of vascularization (blood vessel) formation is more found in the treatment of honey and MEBO ($p<0.05$) (Figure 5). But when compared between honey and MEBO, the administration of honey is stronger to stimulate significantly ($p<0.05$) blood vessel growth in second-degree burns in rats. Honey consists of reducing sugars, sucrose content and certain acidity levels. Forest honey from Apis dorsata bee species from Bima Regency contains active saponin substances, while white brand X honey from Mataram City contains flavonoids[21].

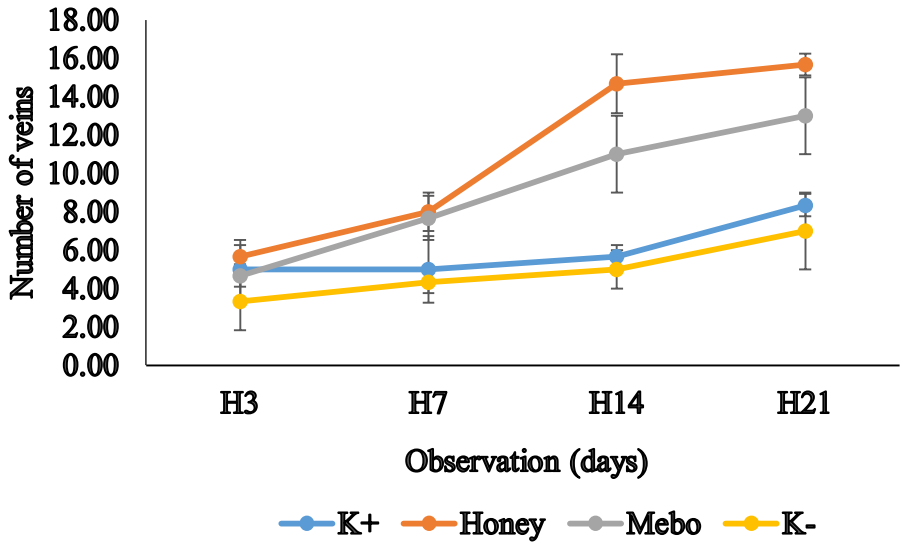

Figure 4. Number of blood vessels on healing day and administration of treatment in the study.

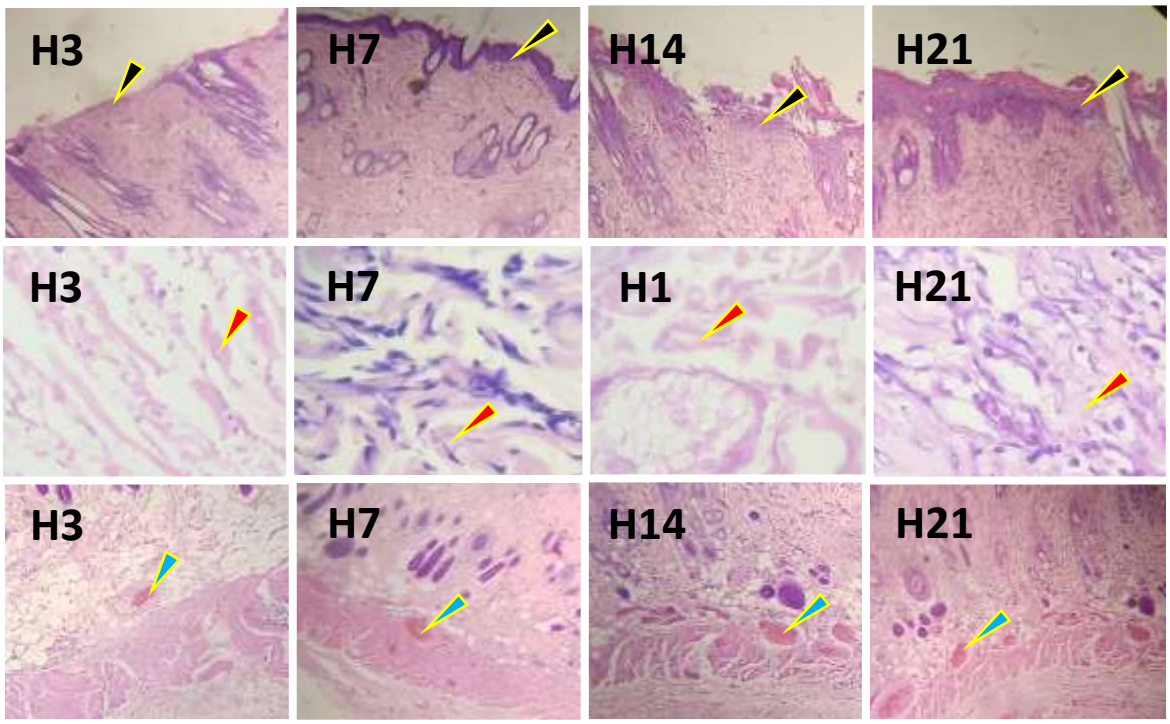

Figure 5. Cross section of skin in second degree burns rats for the treatment of honey from observations 3, 7, 14 and 21 days. Description: black arrow = Stratum corneum (epithelation), red arrow = collagen fiber, green arrow = blood vessel

Discussion

This is because some of the compounds contained in honey have the ability to accelerate tissue regeneration, neovascularization, re-epithelialization, stimulate fibroblasts and collagen formation in burnt trauma and have an antimicrobial effect that will suppress microorganisms that can slow wound healing. According to [(14),] honey is a sweet liquid that is processed by bees which comes from starch or flower pollen and by bees is used as a raw material called nectar. Nectar is obtained in plant cells. Honey bees collect honey in the nest by storing a pollen. Since thousands of years ago until now, honey has been known as one of the natural food or beverage ingredients that have an important role in life.

Honey is one good source of food. Amino acids, carbohydrates, proteins, several types of vitamins and minerals are nutrients in honey that are easily absorbed by the body's cells. A number of minerals contained in honey such as magnesium, potassium, potassium, sodium, chlorine, sulfur, iron and phosphate. Honey also contains vitamins, such as vitamin E and vitamin C and vitamins B1, B2 and B6. In addition, there are also smaller elements, namely: 1) Pigment substances in the form of carotene, chlorophyll, and a number of chlorophyll, and xanthophyll derivative elements; 2) the aroma elements contained are tryptophan, aldehydes, alcohols, and esters; 3) sugar alcohol compounds namely mannitol, dulcitol, tannin and acetylcholine; 4) enzymes in honey, namely invertase, diastase, glucose, oxidase, catalase, phosphatase, and peroxidase; 5) antibiotic and antiviral substances, namely polyphenols and glycosides; 6) vegetable hormones, estrogen-derived hormones, prostaglandin, activating elements of reproductive organs in males and females[15.]

Honey is a naturally occurring sweet substance produced by honey bees from flower bloom nectar, plant secretions or plant-sucking insects. The main sugar is combined fructose (38%) and glucose (31%), while sucrose is found in low concentrations (<8%). Honey also contains small components such as minerals, proteins, amino acids, enzymes, vitamins, and many phenolic compounds. The composition of these compounds varies greatly according to the source of interest in the collection of nectar, seasonal and environmental factors as well as processing, handling, storage and climatic conditions[16].

The number of cells that proliferate and cause thickening of epithelial cells is inseparable from the lack of interference from bacterial cells that can kill epithelial cells (necrosis). Honey can prevent and build bacterial cells in rat burns because it can produce hydrogen peroxide (H_2O_2), which damages the bacterial cell wall and causes bacterial cell contents to come out which eventually kills the bacterial cell. Honey is recognized as a topical antimicrobial agent that is efficacious in the treatment of burns and wounds. The antimicrobial activity in some honey depends on the endogenous hydrogen peroxide content. The level of hydrogen peroxide honey can function as a specific biomarker related to honey and can predict and assess the effects of honey therapy. Using the broth microdilution test, I analyzed the antibacterial activity of 42 Canadian honey against two bacterial strains: *Escherichia coli* (ATCC 14948) and *Bacillus subtilis* (ATCC 6633). MIC90 and MIC50 were formed from a dose-response relationship between antibacterial activity and honey concentration. Canadian honey shows moderate to high antibacterial activity in both bacterial species. Both species, MIC90 and MIC50 revealed that honey showed selective growth inhibitory activity against E. coli, and this activity was strongly influenced by endogenous H_2O_2 concentrations. Bacillus subtilis activity was slightly significantly correlated with the presence of H_2O_2. Removal of H_2O_2 by catalase reduces the antibacterial activity of honey, but the enzyme cannot completely break down endogenous H_2O_2. At the 25% -30% "residual" H_2O_2 significantly correlates with the antibacterial activity of honey residue against E. coli. These data indicate that all Canadian honey studied has antibacterial activity, with a higher selectivity to *E. coli* than *B. subtilis*, and that this antibacterial activity correlates with the production of hydrogen peroxide in honey. Therefore, the level of hydrogen peroxide in honey is a strong predictor of antibacterial activity[18].

The role of TGF-β (Transforming Growth Factor β) as a growth factor in which platelet production is degranulated when the closure of damaged blood vessels occurs. In inflammatory conditions, TGF-β and PDGF (Platelet Derivate Growth Factor) convert fibrinogen to fibrin, then become the fibrin matrix (gel). The fibrin matrix is needed as a bridge for cell migration in subsequent wound healing conditions. In addition, TGF-β also attracts inflammatory cells such as leukocytes (neutrophils, monocytes), and macrophages into the fibrin matrix that fills the wound gap. TGF-β also induces the migration of fibroblast cells into the fibrin matrix. Under proliferation conditions, the fibrin matrix will be replaced by granulation tissue. There are 3 types of granulation tissue cells acting independently in the arrangement of granulation tissue, namely fibroblast cells, macrophages, and endothelial cells. These cells form the new extracellular matrix and blood vessels. During the process of making extracellular matrix, fibroblast cells are a source of collagen production until the extracellular matrix covers the wound gap and provides a bridge for keratinocyte migration. Macrophages produce growth factors such as PDGF and TGF-β which induce fibroblasts to proliferate, migrate, store extracellular matrix, and stimulate endothelial cells to form new blood vessels. The extracellular matrix formed is type 3 collagen which will be replaced with type 1 collagen during the remodeling phase (Figure 5). Saponin contained in honey activates the TGF-β signaling pathway by increasing TGF-β receptor bonds with TGF-β ligands, resulting in an increase in TGF-β receptor expression. This activates more TGF-β. TGF-β activation causes more numbers of fibroblasts to migrate to the wound gap, resulting in an increase in TGF-β proliferase. Fibroblasts are producers of collagen and are stored in the extracellular matrix. As is well known, the extracellular matrix is a collagen pile, with the entry of new collagen produced by fibroblasts, old collagen and

new collagen overlapping which causes thicker collagen in the extracellular matrix. This causes the wound to heal faster.

Conclusions

Based on the analysis of the results of the research and discussion based on the references that have been made, conclusions and suggestions can be taken as below.

1. The contraction process of wound closure observed macroscopically on honey was not significantly different ($p>0.05$) with MEBO for second degree burns in rats.
2. Epithelialization with honey and MEBO was not significantly different ($p>0.05$) in second degree burns in rats.
3. The formation of collagen fibers with honey was denser than MEBO significantly ($p<0.05$) in second degree burns in rats.
4. The amount of vascularization formation with honey was more than MEBO significantly ($p<0.05$) in the healing of second degree burns in rats.

Conflict of interest

"Competing interests: No relevant disclosures".

References

1. Moenadjat, Y. 2009. Practical Clinical Knowledge of Burns. Second edition. Jakarta: Faculty of Medicine, University of Indonesia. Page 1-6
2. Sjamsuhidajat R., De Jong W.2009. Burns. Textbook on Surgery. Revised Edition. Pages 81-85
3. Peck, M.D. 2011. Epidemiology of Burns Throughout The World. Part 1 : Distribution and Risk Factors. *Burns : Journal of the International Society for Burn Injuries.* 37(7):1087-100.
4. Herndon, D (ed.). 2012. Chapter 3 :Epidemiological, Demographic, and Outcome Characteristics of Burn Injury.*Total Burn Care*.(Edisi ke-4). Edinburgh : Saunders. Hlm 23.
5. Rowan MP, Leopoldo C. Cancio, Eric A.Elster, David M. Burnmeister, LIoyd F. Rose, Shanmugasundaram Natesan, Rodney K. Chan, Robert J. Christy, Kevin K.Chung 2015. *Burn Wound Healing and Treatment: Revive and Advancement*.19:243
6. Prasetyo, A.T dan E. Herihadi. 2013. The Application of Moist Exposed Burn Ointment (MEBO) and Binahong Leaves in Treating Partial Thickness Burn : A Case Report. *Jurnal Plastik Rekonstruksi*. 2(4):142-143.
7. Allam, A.M., W. Mostafa, E. Zayed & J. El-Gamaly. 2007. Management of The Acute Partial Thickness Burn Hand Moist Exposed Burn Ointment or Silver Sulvadiazine Cream Both Combined With a Polyethylene Bag. *Annals of Burn and Fire Disasters*. 20(3):144-147.
8. Jewo, P.I., I.O. Fadeyibi, O.S. Babalola, L.C. Saalu, A.S. Benebo, M.C. Izegbu & O.A. Ashiru. 2009. A comparative Study of The Wound Healing Properties of Moist Exposed Burn Ointment (MEBO) and Silver Sulphadiazine. *Annals of Burns and Fire Disasters.* 22(2):79.
9. Kanyaluck Jantakee, Yingmanee Tragoolpua. Activities of Different Tipes of Thai Honey on Pathogenic Bacteria Causing Skin Disease, Tyrosinase Enzyme and Generating Free Radicals. *Journal of biologica Research NCBI*, 2015:48(1):4
10. Isrofah, Sagiran, Moh. Afandi, Effectiveness of Ointment of Binahong Leaf Extract (Anredera Cordifolia (Ten) Steenis) Against the Process of Healing Thermal Level 2 Burns in White Mice (Rattus Novergicus). Muhammadiyah Journal of Nursing, 27-39
11. Nagaoka, T., Y. Kaburagi, Y.,Hamaguchi., M. Hasegawa., K. Takehara. (2000) . Delayed Wound Healing in The Asence of Intercellular Adhesion Molecule-1 Or L-Selectin Expression. Am Journal Pathology. 157: 237-47
12. Hosseini, S.V., H. Niknahad., N. Fakhar., A. Rezaianzadeh., D. Mehrabani. 2011.The Healing Effect of Mixture of Honey, Putty, Vitriol and Olive oil in Pseudomonas aeroginosa Infected Burns in Experimental Rat Model. *AsianJournal of Animal and Veterinary Advances.* 6(5):572-79.
13. Panglinawan, Rey., Colic, Miodrag., Simon, Maryline. (2008). A Comparative Study of The Influence of Diff eren Pressure Levels Combined with Various Wound Dressingson Negative Pressure

WoundTherapy(NPWT) Driven Wound Healing.Medela AG, Healthcare, Switz erland.Salami, Ayodeji A., Imosemi, Innocent O.,Owoeye, Olatunde O. 2006.

14. Arif Mz. 2017 Effect of Honey on Burns. Medal 7 (5), 71.
15. Saqa M. 2010. Treatment with honey. Jakarta: Al-Kautsar Library
16. Hussein S.Z, K.M. Yusoff, S. Makpol, Y.A. Mohd Yusof. 2014. Does Gamma Irradiation Affect Physicochemical Properties of Honey ?. Clin Ter; 165 (2): e125-133. doi: 10.7471 /CT.2014.1695.
17. Nurfitriani, R. 2014. Effect of the use of honey and propolis combination cream on histology of epithelial thickness healing incision wounds on the skin of white mice (*Rattus norvegicus*). Scientific work. Faculty of Medicine and Health Sciences, Muhammadiyah University of Yogyakarta.
18. Brudzynski K. 2006. Effect of hydrogen peroxide on antibacterial activities of Canadian honeys. Can. J. Microbiol. 52: 1228–1237.
19. Aulia, A.F. 2014. Effect of binahong leaf extract ointment (*Anredera cordifolia* (Tenore) Steenis) on granulation tissue formation in rat burns (Sprague Dawley) (long preliminary study of exposure to 10 second burns with iron plate), http://repository.uinjkt.ac.id/dspace/bitstream/123456789/26176/1/SYIFA%20QURROTU%20AINI-fkik.pdf.
20. Buba F, Gidado A, Shugaba A. 2013. Analysis of Biochemical Composition of Honey Samples from North-East Nigeria. Biochem Anal Biochem 2: 139. doi: 10.4172/2161-1009.1000139.

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