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Comparison of Antibacterial Effectiveness and Length of Healing between Honey and MEBO in Degree-II Burns at the White Rat (*Rattus norvegicus*)

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Abstract : Healing Second degree burns can be affected by the presence of bacteria. This research reveals the role of honey and MEBO in removing bacterial effects on healing. Pure experimental research method with a completely randomized design (CRD). There were 4 treatments, which were positive, negative, honey and MEBO treatment, each of which was given to rats with second degree burns. Wound healing and other parameters were observed on 7th, 14th and 21st days. The result was that the administration of honey as an antibacterial drug was very effective and gave healing on the 21st day to white rats. Giving MEBO as an antibacterial drug is very effective and provides healing on the 21st day to white rats. Giving honey is more effective than MEBO as an antibacterial drug and healing burns on 21st day against white rats.
Key words : antibacterial, second degree burns, honey, MEBO.

Introduction

Burns are tissue damage or loss that can be caused by heat (fire, liquid / hot fat, hot steam), radiation, electricity, chemistry[1]. Until 2004, 11 million burns needed medical care worldwide and caused 300,000 deaths. This makes burns the cause of the fourth major injury after motor vehicle accidents, falls, and acts of violence. About 90% of burns occur in developing countries. This is partly due to excessive population density and unsafe cooking conditions[2]. Overall, almost 60% of fatal burns occur in Southeast Asia with an incidence rate of 11.6 per 100,000 inhabitants[3].

Burns most often occur at home and most are found in second degree burns[4]. In second degree burns, the skin dermis is damaged, but there is still a layer of skin epithelium. With this epithelial layer, the wound will heal within 2 to 3 weeks. The feared factor in the healing process of this burn is infection. Prevention of infection can be done with topical treatment or even oral antibiotics[5].

One of them is Moist Exposed Burn Ointment (MEBO), an oil-based ointment containing sesame oil, beta-sitosterol, beberine, and a small amount of other plant ingredients developed at the Chinese National Science and Technology Center in Beijing, China in 1989 which has proposed as an ideal treatment for burns[5].

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MEBO began to favor its use in the treatment of burns because the healing process of burns was relatively fast[6]. MEBO is believed to protect wounds from infection and improve wound healing without causing side effects[7]. The use of MEBO turned out to have a barrier in the form of relatively high prices compared to other types of burns[6].

The public does not understand that in fact many researchers have researched scientific materials that have been tested for healing wounds, including burns. One of them is honey[8]. Honey therapy has been tried for the treatment of burns in several clinical trials and the results are promising. The benefits of honey have been known for centuries and recent research has also shown that honey helps accelerate wound healing rather than conventional therapy[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. In addition, the price of honey itself 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[8].

Material and Methods

Animal Experiment

Male adult white-rat (*Rattus norvegicus*) obtained from the Faculty of Biology, University of North Sumatra. The rats sampled weighed around 150-250 grams and numbered 30 for 3 treatment groups (positive control, honey, MEBO) with each group consisting of 10 rats. Rat research received permission from the Health Research Ethics Committee of the Faculty of Mathematics and Natural Sciences, Number 0256/KEPH-FMIPA/2018.

Procedure

Before the treatment of all laboratory rats, 30 white rats were adapted at the Pharmacology Laboratory of the Faculty of Medicine, Universitas Sumatera Utara for 2 weeks. After the adaptation period, the rats are separated into one cage containing one rat. After that, burns on the skin of the rat were given by means of the hairs of rats 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 for 2 seconds. After the burns were formed, honey and MEBO were applied to the skin of the rat honey treatment group and MEBO. Whereas in the positive control group, the treatment of burns only used aquadest.

Procedure for Degree II Burns Treatment

Handling of burns must be careful so cleaning must be done first using aquadest before giving MEBO or honey ointment. On applying the burn medicine can be given twice a day. The steps for handling burns are prepared gauze and arranged the position of rats to facilitate action. Smeared with the wound with gauze that has been moistened with honey Kabanjahe livestock (Efi honey) 2-3 mm thick 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 positive control treatment group, the wound was covered with 3 layers of sterile gauze moistened with distilled water, squeezed, 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 aquadest control group was carried out for ± 21 days. Microbiological observations and testing of bacteria were carried out after treatment for 7 days, 14 days to 21 days.

Sterilization of Tools

The tools to be sterilized are washed and dried first, petri dishes wrapped in parchment paper, for glassware (test tubes, measuring cups, Erlenmeyer), their mouths are covered with sterile cotton wrapped in sterile gauze and wrapped in parchment paper. Then sterilized everything in an autoclave at 121^{o}C, a pressure

of 15 lbs for 15 minutes. Tweezers, ose needles, and object glass are sterilized by flambir. Laminar flow water is sterilized by turning on the UV light for 5 minutes. Aseptic cabinets are cleaned of dust and then sprayed with 70% alcohol, left for 15 minutes[5].

Media Preparation

Media Fluid Thyoglycollate (FT)

Twenty nine gram tioglycollate powder to be dissolved in 1 L of aquadest in erlenmeyer, heated to homogeneous, then sterilized in an autoclave at 1210C at a pressure of 15 lbs for 15 minutes. After sterile poured into a test tube as much as 15 mL[10].

Media of Blood Agar (BA)

Fourty gram of Blood Agar powder dissolved in 1 L aquadest and heated to boiling while stirring, then sterilized by autoclaving at 121 pada C, a pressure of 15 lbs for 15 minutes. After being sterilized, 50 mL of fresh lamb blood was added, mixed until smooth, then poured into 15 mL petri dishes.

Sampling

Samples taken in the form of swab / smear from secretions of burns that have been infected in white rats. Burns in rats are cleaned first with 70% alcohol and allowed to stand for ± 10 minutes, then burns are pressed slowly so that the liquid under the skin is obtained properly, then the liquid is taken by using sterile sticky cotton twice for taking. The first smear was fixed to the slide for Gram staining and the second smear was put into four thioglycolate media, tightly closed and the test continued in the microbiology laboratory.

Bacterial Isolation

Swab samples from burns were taken using sterile sticky cotton and then inoculated into Nutrient Broth (NB) media, then incubated for 18-24 hours at 370C. Then planted in a petri dish containing Nutrient Agar (NA) media.Then incubated for 24 hours at 370C.After that identification of bacteria.

Identification of Bacteria[10,11].

Gram staining

Gram staining is used to determine the type of Gram positive bacteria and Gram negative bacteria. Gram staining is tested by way, the object glass is cleaned with alcohol so that it is free of fat, then fixed above the spirits lamp to dry, then given a drop of physiological NaCl. Bacteria from the media are taken with ose needles, placed on physiological NaCl droplets, then mixed evenly. Let it dry in the air for a while and fix it on fire. Then drops 2-3 drops of violet Crystal solution and leave for 1 minute, then rinse with running water. After that, one drop of lugol solution was poured and left for 1 minute, then rinsed with running water and dried, the preparation was rinsed with 96% alcohol for 30 seconds then washed with running water and dried. Finally it is dripped with safranin solution and left for 1 minute then rinsed with running water and dried. After that this preparation was observed under a microscope. Purple for gram positive bacteria and red for gram negative bacteria[11].

Catalase Test

Catalase testing is useful in identifying groups of bacteria that can produce the catalase enzyme so that it can be distinguished between aerobic and anaerobic bacteria. Catalase test was carried out by means of a drop of 3% H_2O_2 on the object glass, then a bacterial colony was added and immediately observed the decomposition of hydrogen peroxide. The results are positive when producing the enzyme catalase which is characterized by the formation of air bubbles. And the results are negative if there are no air bubbles[11].

TPC (Total Plate Count) Method

Test the Total Plate Count or also called the TPC (Total Plate Count) using solid media with the end result in the form of colonies which can be observed visually in the form of numbers in the colony (CFU) per

ml / g or colony / 100ml. The principle of testing the Total Plate Figures is the growth of mesophyll aerobic bacteria colonies after the samples are inoculated on the agar plate media by pouring and incubating at the appropriate temperature.

Before the bacteria are grown in agar media, the sample dilution is done first using a physiological solution. A sample of 1 g was added 9 ml of 0.9% NaCl, then homogenized to obtain a suspension with a dilution of 10-1. Then take 1 ml of the dilution sample with a volume pipette and put it into a test tube containing 9 ml of 0.9% NaCl (10-2 dilution). Then do the same treatment until 10-3 and 10-4 dilutions are obtained. Samples that have been diluted to 10-4, taken as much as 1 ml and dropped on an empty petri dish. Subsequently poured Nutrient Agar (NA) media into each petri dish, mixed until homogeneous and then allowed to solidify. After solidifying, the petri dishes were incubated at 370C for 24-48 hours[11].

Calculation of Bacterial Colonies

After incubation, all colonies that grow on the media are counted. Colony units are determined based on the number of colonies per 10 grams of sample. Ideally the number of colonies per petri dish that can be calculated is between 30 - 300 CFU (Colony Form Unit). Large, small, creeping colonies are ascribed to one bacterium. Calculations can be done manually by giving a dot using a marker on a petri dish or by using a colony counter. Calculation of TPC (Total Plate Count) is expressed as the number of bacterial colonies calculated by multiplying the dilution factor[11].

Data analysis

The results of the last study were analyzed whether it had a normal distribution ($p > 0.05$) or not statistically with the Shapiro-Wilk normality test because the number of samples was ≤ 50. Then Levene test was conducted to find out whether two or more data groups had the same variance ($p > 0.05$) or not. If the variance of the data is normally distributed and homogeneous, it will be continued with the repeated ANOVA parametric test method. If it does not meet the parametric test requirements, a transformation will be carried out. If the ANOVA test produces a p value of <0.05, it will be followed by conducting a post hoc LSD analysis to see differences between treatment groups. If the transformation results do not meet the requirements, the Friedman test is used and followed by the Wilcoxon test.

Results and Discussion

Antibacterial Test Results

The presence of this type of bacteria was seen by catalase testing in each group of observation days. Negative marking shows the absence of bacteria in rat skin burns and the number of types of bacteria is indicated by a percentage. These results mark the results of antibacterial tests with various treatment groups marked by the percentage of several types of bacteria for burns in research rats within a few days of observation listed in Table 1 below.

Table 1. Antibacterial test results of several treatment groups for several days of observation.

	7 days (%)			14 days (%)			21 days (%)		
Treatment	**Klebsiella pneumonia**	**Candida albicans**	**Pseudomonas aeruginosa**	**Klebsiella pneumonia**	**Candida albicans**	**Pseudomonas aeruginosa**	**Klebsiella pneumonia**	**Candida albicans**	**Pseudomonas aeruginosa**
K+	100,00	83,33	(-)	83.33	83.33	100	100	83.33	(-)
K-	(-)	50,00	100	(-)	83.33	100	(-)	66,67	100
Honey	50	83,33	(-)	50	83.33	(-)	(-)	(-)	(-)
Mebo	100,00	100,00	(-)	(-)	100	(-)	(-)	33,33	(-)

In general, Table 1 illustrates that the types of bacteria found in skin burns of research rats were *Klebsiella pneumonia*, *Candida albicans* and *Pseudomonas aeruginosa*. Its presence in observations of days 7, 14 and 21 is not always present, because of the antibacterial effect of honey and MEBO.

The antibacterial test results in Table 1 explain that giving honey to healing burns gave the best results in suppressing the presence of bacteria that prevented healing of rat burns on day 21, followed by the use of MEBO. But on the 14th day, MEBO was used to kill the presence of bacteria faster, especially the bacteria *Klebsiella pneumonia* and *Pseudomonas aeruginosa*.

Honey is a natural sweet ingredient produced by honey bees from flower nectar[12]. Honey content varies and depends on various factors such as the source of the plant and the species of bee that produce it, but generally consists of 75% - 80% carbohydrates, 17% -20% water, and 1% -2% minerals and other organic materials. Honey supports metabolism and digestion, increases energy, helps sharpness of vision, increases weight, as an antioxidant, helps in healing bronchial asthma and urinary tract abnormalities, prevents bacterial infections and as an antiseptic that can help wound healing, even honey can be potent bactericidal in some life-threatening antibiotic resistant cases[13].

Wound healing in the Moist Exposed Burn Ointment (MEBO) group looked faster (Table 2). This is because MEBO is antibacterial as seen in Table 1. Bacteria can cause infection and slow the occurrence of wound healing. In accordance with the opinion of Prasetyo and Herihadi[5], that Moist Exposed Burn Ointment (MEBO) can accelerate healing, inhibit bacterial growth, have analagetic effect, and reduce burn scars

Re-epithelialization is affected by levels of apoptosis, proliferation and migration[14,15]. In vitro injury of primary keratinocytes occurs and is a bacterial effect on apoptosis of keratinocytes in the area near the edge of a burn. Klebsiella pneumonia, *Candida albicans* and *Pseudomonas aeruginosa* increase apoptosis several times at the edges of burns. *Candida albicans* causes greater epithelial cell apoptosis than other bacteria. This was seen in the MEBO treatment of *Candida albicans* still surviving on day 21 but in the honey group Candida albicans survived on day 14 (Table 1).

Re-epithelialization of the bald skin surface is associated with the presence of bacterial proteases[16,17]. Protease can inhibit serine and cysteine which play an important role in cell proliferation. So that it inhibits bacterial cell growth so that it supports cell proferation or rapid wound healing.

Good wound healing in honey and MEBO was also caused because both of them killed the Pseudomonas aeruginosa bacteria on the seventh day observation. Pseudomonas aeruginosa can inhibit the migration of epithelial cells for wound healing. According to Kimberly et al.[18], that secretory (*eg*. LPS / lipopolysaccharide) of 95% *Serratia marcescens*, 71% of *Pseudomonas aeruginosa*, 29% of *Staphylococcus aureus* strains, and other bacterial species inhibited epithelial cell migration. This information presents the interactions of new host-pathogens with implications for infections where bacteria have an impact on wound healing and provides evidence that LPS secreted is a key factor in the inhibitory mechanism. According to Klemm et al.,[19], Molteni et al.,[20] and Boonen et al.,[21], LPS in the form of endotoxin or gram negative bacterial toxin is located on the outer membrane of the cell wall which in certain circumstances is toxic in certain host. This lipopolysaccharide is called endotoxin because it binds to bacteria and is released when microorganisms undergo cell lysis or rupture.

Bacterial Total Plate Count Test Results on Burns

The results of measuring the total bacterial plate count in rat burns are listed in Table 2 below. The number of bacterial colonies decreases with time of research observation. Especially the administration of honey which can reduce the number of bacterial colonies to 0, but not significantly different from the results of MEBO giver ($p>0.05$).

Table 2. Total number of bacterial colonies in various treatment groups on observations of days 7, 14 and 21.

Groups	**Obsevations (days)**		
	7	**14**	**21**
K+	67.67 ± 2.08^{d}	88.67 ± 1.53^{e}	49.00 ± 2.00^{c}
K-	47.00 ± 4.00^{c}	52.67 ± 3.51^{c}	34.00 ± 4.58^{b}
Honey	47.33 ± 3.51^{c}	35.67 ± 5.13^{b}	0.00 ± 0.00^{a}
MEBO	62.33 ± 4.51^{d}	32.33 ± 6.51^{b}	4.67 ± 5.03^{a}

Description: $p^{a,b}<0.05$; post hoc Duncan analysis between treatment groups and observation days.

Giving honey in suppressing the number of bacteria in rat burns is very good although not significantly different from the provision of MEBO. Honey contains lots of vitamins, minerals, antioxidants and various antibacterial substances.

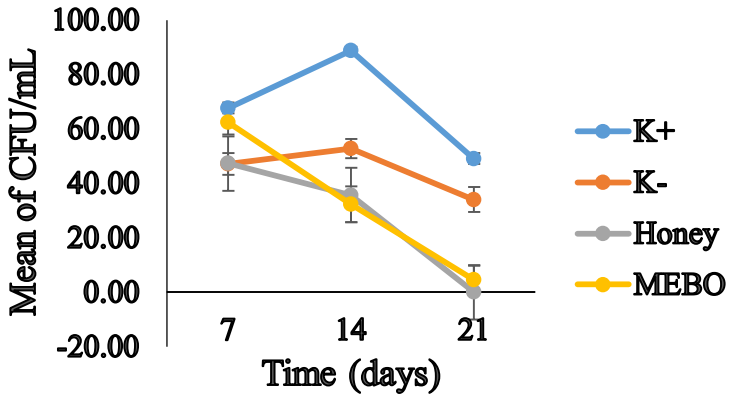

Fig. 2.Average CFU / mL of rat burns per observation of days 7, 14 and 21.

Fig. 2 shows the inhibition of bacterial growth in the honey treatment group and MEBO starting from observations on days 7, 14 and day 21. Giving honey secretes H_2O_2, which disrupts the function of proteases released by bacterial cells. Fibroblast cell proliferation activity increases and eventually accelerates the healing of burns in rats.

The mechanism of honey as an antibacterial can be classified directly and indirectly[21]. The mechanism is directly based on the ability of the honey component to kill bacteria. Indirect mechanism is the antibacterial response of the host stimulated by honey to bacteria. The mechanism is directly the mechanism for the formation of hydrogen peroxide (H_2O_2), high osmolality, low pH, non-peroxide factors, and phenol. Indirect antibacterial mechanisms include lymphocytes and the production of antibodies, cytokines and immune responses, and nitric oxide[21].

Long Healing Effectiveness Test Results

The results of the long-term effectiveness test for wound healing associated with the percentage of wound closure are listed in Table 1 below.

Table 2.Results of percentage healing analysis in each treatment for one day, seven days, 14 and 21 days.

Treatment	Percentage of Wound healing (%)			
	H1	**H7**	**H14**	**H21**
K-	0.00 ± 0.00^{a}	10.48 ± 10.38^{b}	15.87 ± 10.25^{ab}	75.53 ± 11.87^{b}
K+	0.00 ± 0.00^{a}	-13.87 ± 12.21^{a}	0.29 ± 17.51^{a}	53.18 ± 3.29^{a}
Honey	0.00 ± 0.00^{a}	6.32 ± 12.61^{b}	16.04 ± 9.85^{ab}	78.57 ± 10.53^{b}
MEBO	0.00 ± 0.00^{a}	17.26 ± 10.93^{b}	23.31 ± 11.84^{b}	88.74 ± 3.04^{c}

Description: $p^{a,b}<0.05$ with Duncan's post hoc analysis between treatments.

The percentage of healing of rat burns from the first day (H1) to day 21st (H21) was seen as close to 100%, both the Control (-), Control (+), honey and MEBO groups. Based on the treatment groups at each observation time (H1, H7, H14 and H21) there were significant differences ($p<0.05$) especially in H14 and H21. Whereas in Fig. 2 there is a graph of the percentage increase in healing (%) for all treatment groups.

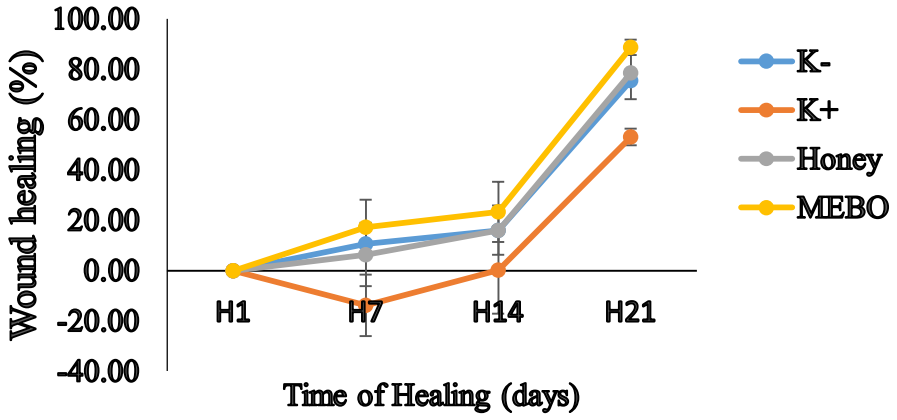

Fig. 2.Average healing of burns (%) during observation (days) of the study.

Based on Table 2 and Fig. 2, healing of burns or closure of burns (%) occurs in H14 and H21. Giving MEBO to burns heals faster than using honey significantly ($p<0.05$). This is different from the results of the Alawiyah[21]study, which showed that honey has a speed of healing burns that is not much different from MEBO which was shown in a post-hoc paired wise comparison test ($p>0.05$).

Giving honey and MEBO in dealing with burns is very good and based on the time of measurement formed from H14 and H21. Honey has several sources of nutrients that are rich in amino acids, carbohydrates, proteins, vitamins and minerals that play a role in accelerating the healing of the skin. Nutrient intake will encourage cell proliferation, migration and inhibit protease activity (bacterial products) that cause cell death or prevent new cell proliferation. According to Molan and Rhodes[25], the physical properties of honey also accelerate the healing process: its acidity increases the release of oxygen from hemoglobin, making the wound environment less favorable for destructive protease activity, and the high osmolarity of honey secretes fluid from the wound to create lymph secretions. Honey has broad spectrum antibacterial activity, but there are many different variations between different honey. There are 2 types of antibacterial activity. In most honey this activity is related to hydrogen peroxide, but quite a lot of bacteria from this are not activated by the enzyme catalase which is in the blood, serum, and wound tissue. In manuka honey, this activity is caused by methylglyoxal which is not deactivated. Manuka honey used in wound care products can resist dilution with a large amount of wound exudates and still maintain sufficient activity to inhibit bacterial growth. There is also good evidence for honey which also has bioactivity that stimulates the immune response (thus encouraging tissue growth for wound repair), suppresses inflammation, and causes rapid autolytic debridement. There is clinical evidence for this action, and research provides a scientific explanation for this action. Healing by MEBO is caused by the abundant content of the active ingredients in MEBO such as fatty acids, amino acids and vitamins.

MEBO is an ointment whose composition consists of several herbal ingredients such as Radix *scutellaria*, Cortex *Phellodendri* and Rhizoma *coptidis* which contain sesame oil (oil), beeswax, 18 amino acids, 4 fatty acids, 7 polysaccharides and vitamins that function to help the skin do re-epithelialization, providing nutrition and helping to speed up cleaning of necrotic tissue besides that it also functions to facilitate the exfoliation of dead tissue in liquefaction, triggers the regeneration process, while acting as a nutrient for the wound healing process[26]. Burns that involve all the dermis and part of the dermis. Manifestations that appear on the skin in the form of redness, there are also, edema, and severe pain. This burn can heal within 7-21 days. At the second degree there are 2 types namely[27].

Based on the results and discussion of the research that has been conducted regarding the administration of honey and MEBO to burns in rats can be concluded as follows: (a) Giving honey as an antibacterial drug is very effective and provides healing on the 21st day to rats. (b) The administration of MEBO as an antibacterial drug is very effective and provides healing on the 21st day to rats. (c) Giving honey is more effective than MEBO as an antibacterial drug and healing burns on 21st day against rats.

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