

Effectiveness of Indonesian Honey on the Acceleration of Cutaneous Wound Healing: An Experimental Study in Mice

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(Full title) EFFECTIVENESS OF INDONESIAN HONEY TOWARD ACCELERATION
OF CUTANEOUS WOUND HEALING: AN EXPERIMENTAL STUDY IN MICE

(Short title) Effectiveness of Indonesian honey on wound healing

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Abstract

The purpose to investigate the effectiveness of Indonesian honey in wound healing in comparison with Tegaderm hydrocolloid dressing and Manuka honey. Three groups of male mice were treated to produce two circular full-thickness skin wounds on the dorsum. They were then randomly allocated to receive daily Indonesian honey, Manuka honey or hydrocolloid as a control for treatment application. Macroscopic findings were observed from day 0 to 14 after wounding. Microscopic findings on days 3, 7, 11 and 14 after wounding were obtained. The ratios of wound areas for honey groups on day 3 were smaller than those of the control group. Wound areas of honey groups gradually decreased to almost the same wound area as the control group on day 14, while the wound area of the control group peaked on day 5 and rapidly decreased until day 14. On day 3, myofibroblasts and new blood capillaries in wound tissue of honey groups were observed, but not in the control group. After day 7, microscopic findings were almost the same among all groups. These results indicate that Indonesian honey is almost as effective for wound healing as Manuka honey and Tegaderm hydrocolloid dressing.

Keywords: Indonesian honey, Manuka honey, Myofibroblast, New blood capillary, Tegaderm hydrocolloid dressing,

INTRODUCTION

Wound healing is a dynamic physiological process initiated and influenced by many factors. The process can be divided into four stages: hemostasis, inflammation, proliferation (granulation, contraction and epithelialization) and remodeling.¹

A lot of modern dressings such as hydrocolloids, gels and foams have been commonly used in clinical settings recently. However, they are not cheap in developing countries. As an alternative, traditional medicine, especially honey, can be used as a topical dressing for wound care.

Honey is a byproduct of flower nectar and the upper aero-digestive tract of honey bee, which is concentrated through a dehydration process inside the beehive.² The use of honey as a medicine has been known for tens of thousands of years, for example, in the treatment of diseases of digestive organs, eye and cough.³ In addition, honey can be used as a topical therapy for burns, infections and skin ulcers.⁴

To date, many results of research have been reported showing that honey is effective for the treatment of wounds, both clinically and in the laboratory. Furthermore, there have been several results of research showing that honey is very effective at increasing granulation tissue and collagen, as well as epithelialization, when it is used as a topical therapy in wound care.⁵ Another study has reported that honey can also decrease wound area.⁶ In general, pure commercial unboiled honey is composed of approximately 40% glucose, 40% fructose, 20% water, amino acids, the vitamin biotin, aminonicotinic acid, folic acid, pantothenic acid, pyridoxine, thiamine, the enzymes diastase invertase, glucose oxidase and catalase, and the minerals calcium, iron, magnesium, phosphorus and potassium.⁶ Another study showed that honey is a mixture of sugars (about 40% fructose, 30% glucose and 10% maltose) including

oligosaccharides, minerals, carbohydrates, enzymes and phytochemicals such as flavanoids, as well as ferulic and caffeic acids.⁷

The purpose of this study is to investigate the effectiveness of Indonesian honey in accelerating wound contraction, granulation, epithelialization and myofibroblast activity in wound healing in comparison with a modern dressing, Tegaderm hydrocolloid dressing, and Manuka honey.

MATERIALS AND METHODS

Animals

Thirty-six BALB/cCrSlc male mice aged 8 weeks and weighing 22.0-23.8 g were used. They were caged individually in an air-conditioned room at $25.0 \pm 2.0^\circ\text{C}$ with lighting from 08:45 to 20:45. Water and laboratory chow were given ad libitum. The experimental protocols were in accordance with the Guidelines for Care and Use of Laboratory Animals of Kanazawa University, Japan.

Honey

Two types of honey were used: Indonesian pure honey (*Apis dorsata*) and commercial honey, namely, Manuka honey (*Leptospermum scoparium*).

Wounding

Mice were anesthetized with i.p. injection of sodium pentobarbital (0.05 mg/g weight). Two circular (4 mm in diameter) full-thickness skin wounds including the panniculus on both sides of the dorsum of the mouse were made with a sterile disposable biopsy punch (Kai Industries Co. Ltd., Gifu, Japan). The day when wounds were made was designated as day 0. Wounds of the experimental groups, Indonesian honey and Manuka honey groups, were treated with either 0.1 mL Indonesian or 0.1 mL Manuka honey, respectively. Treatments were applied using a 1 mL syringe. All wounds on an individual animal received the same treatment. The wounds to which honey was applied were covered with gauze as a secondary dressing to prevent honey run-off and to absorb exudate that leaked from the surface of the wound. The gauze, 28 mm x 10 mm was got from the sterilization gauze of wound adhesive plaster. The gauze was changed and all wounds were treated with honey every day. Mice were wrapped twice with sticky bandage (Mesh pore tape; Nichiban, Tokyo, Japan) so that the gauze did not slip out of position. Additionally, the process of wound healing was observed once daily from

day 0 to 14 after wounding. Meanwhile, wounds of the control group were covered with hydrocolloid dressing (Tegaderm; 3M Health Care, Tokyo, Japan). All mice were wrapped twice with sticky bandages that were changed every day. Wound areas were traced on polypropylene sheets and photographs were taken every day. Wound areas were evaluated using Scion Image Beta 4.02 (USA) and Adobe Photoshop Elements 6.0 (Japan).

Histological procedure

The mice were euthanized by a massive sodium pentobarbital i.p. injection on day 3, 7, 11 or 14 after wounding. The wounds and the surrounding normal skin were excised for an area of about 15 mm x 15 mm square, stapled onto polypropylene sheets to prevent overcontraction of the samples and fixed in 4% paraformaldehyde in 0.2 mol/L phosphate buffer (pH 7.4) for 12 hours. The samples were dehydrated in an alcohol series, cleaned in xylene and embedded in paraffin to prepare 5 μ m serial sections.

Alternate sections involving the wound center were stained with hematoxylin-eosin (HE) or Azan, and immunohistologically stained with anti- α -smooth muscle actin (α -SMA) antibody. The immunohistological staining was performed as follows. The paraffin of the sections was removed with xylene, then they were washed with 0.01 mol/L phosphate-buffered saline (PBS), pH 7.4, covered with 0.03% hydrogen peroxide to block endogenous peroxidase for 20 minutes at room temperature, rinsed with PBS, incubated in a solution of monoclonal Mouse Anti-Human Alpha Smooth Muscle Actin (Dako North America Inc., CA) for 60 minutes at room temperature, rinsed with PBS, followed by incubation with Dako EnVision+System-HRP Labeled Polymer Anti-Mouse (Dako North America Inc., CA), and washed in PBS. The final reaction product was developed for 5-10 minutes with a 3,3'-diaminobenzidine substrate (Dako ENVISION Kit/HRP (DAB), Dako Japan, Kyoto, Japan). The sections were washed in PBS and then counterstained with hematoxylin and washed again in tap water. Negative controls were obtained by omitting primary antibody.

Counting the numbers of neutrophils, capillaries and myofibroblasts

The numbers of neutrophils, capillaries and myofibroblasts in granulation tissue were counted by observation through a light microscope with 400x magnification. Five to six sections from 3 different mice for days 3, 7, 11 and 14 after wounding were used for the entire area from the center of the wounds. The number is expressed per mm².

Measuring re-epithelialization and thickness of wound tissue

The measurement of wound epithelialization was defined as the length of new epithelium (mm) divided by the length of wound on the day (mm) as determined using Adobe Photoshop Elements 6.0 and Scion Image Beta 4.02. Five to six sections were used from 3 different mice for days 3, 7, 11 and 14 after wounding. The thickness of wound tissue (mm) was measured at the midpoint of the wound on the section using Adobe Photoshop Elements 6.0 and Scion Image Beta 4.02. The thickness of the wound was the length of the perpendicular line to the wound surface from the line linking both panniculus muscles.

Statistical analysis

Data are expressed as mean \pm SD, and were analyzed using JMP® 8.0.1 (SAS, Cary, NC). ANOVA and Tukey-Kramer multiple comparison test were performed. The differences were considered significant at $p < 0.05$.

RESULTS

Macroscopic observation of wound healing

Observation (Figure 1)

On the 2nd to 5th days after wounding, the area surrounding the wound in Indonesian and Manuka honey groups had apparent redness but no edema or smell. This condition was the same as that of the control group, but the wound area in the control group was larger than those of Indonesian and Manuka groups. On day 7, the area surrounding the wound in Indonesian and Manuka honey groups had no redness. The wound areas of both groups were smaller than those on day 0. Granulation and new epithelium were observed. In the control group, granulation and new epithelium were apparent, but the wound area was still larger than those for Indonesian and Manuka honey groups. On day 11, the entire areas of wounds of all groups were covered by new epithelium, and the wound areas of all groups were almost the same. On day 14, wounds of all groups healed with scarring but without infection.

The ratio of wound area (Figure 2)

The ratios of wound areas on day 0 to day 14 after wounding to the initial wound area on day 0 were calculated. There was no significant difference between the wound area of the Indonesian honey group and that of the Manuka honey group throughout the whole period, day 0 to day 14 after wounding. On the other hand, wound area in the control group increased after wounding, peaked on day 5 and then decreased rapidly. While there were significant differences between the control group and the Indonesian and Manuka honey groups from 1 day to 10 days after wounding, on day 11 after wounding the wound areas in control, Indonesian and Manuka honey groups were 0.46 ± 0.15 , 0.42 ± 0.16 and 0.43 ± 0.08 times smaller (on day 11), respectively, than those on day 0 and there were no significant

differences between the control group and the Indonesian honey ($p=0.87$) and Manuka honey groups ($p=0.92$) on day 11 after wounding.

Microscopic observation on days 3, 7, 11 and 14 after wounding

Histological observation

On day 3 after wounding (Figure 3), during the inflammation phase, new epithelium along the wound edge appeared in all groups and expanded towards the center of the wound. The wound floor in the control group expanded more than those in the experimental groups. This may have been due to edema. In the control group, no granulation tissue was observed. Thin collagen fibers stained blue by Azan staining were observed in the wound tissue of all groups. A lot of neutrophils and macrophages, that is, inflammatory cells, were already present in the wound tissue of all groups. New blood capillaries and myofibroblasts containing α -smooth muscle actin stained brown were already observed in the wound tissue of Indonesian honey and Manuka honey groups, but not in the control group. This may indicate that granulation tissue consisting of collagen fibers and new blood capillaries was formed more rapidly owing to the Indonesian and Manuka honey than with the hydrocolloid dressing

On day 7 after wounding (Figure 3), granulation tissue consisting of collagen fibers and new blood capillaries was observed and filled the concave space of the wound in the control group. New epithelium formed along the wound edge and almost covered the wound surfaces in all groups, while new epithelium covered the wound surface of the control group more widely than those of the experimental groups. Collagen bundles in the Indonesian honey group seemed to be thicker than those of the other groups in the granulation tissue. In the Indonesian honey and Manuka honey groups, many myofibroblasts and blood capillaries appeared in granulation tissue, which were more abundant than those on day 3.

Myofibroblasts in granulation tissue of the Indonesian honey group seemed to be more abundant than those in Manuka honey and control groups.

On day 11, the whole wound surface was almost completely covered with new epithelium. All groups exhibited collagen throughout the granulation tissue. Myofibroblasts appeared throughout the granulation tissue in the Indonesian honey group and were more abundant than those in the control group. Myofibroblasts in the Manuka honey group appeared in the granulation tissue and were more abundant at the wound edges, and were also more abundant than those of the control group.

On day 14, the wound surface was completely covered with new epithelium. Tight bundles of collagen filled the whole of the granulation tissue of all groups and produced scars. Myofibroblasts appeared throughout the granulation tissue and underneath the new epithelium in all groups. A few blood capillaries were observed in the scars of all groups.

The number of neutrophils (Table 1)

On day 3, the numbers of neutrophils in the Indonesian honey and Manuka honey groups were larger than that of the control group. There were significant differences between the control group and the Indonesian honey group ($p=0.0003$) and the Manuka honey group ($p=0.0010$). However, there was no significant difference between the Indonesian honey and Manuka honey groups ($p=0.68$).

On day 7, the numbers of neutrophils in Indonesian honey, Manuka honey and control groups decreased compared with those on day 3. There were significant differences between the control group and the Indonesian honey group ($p=0.02$) and the Manuka honey group ($p=0.02$). However, there was no significant difference between the Indonesian honey group and the Manuka honey group ($p=0.99$). After day 7, the numbers of neutrophils in Indonesian

honey, Manuka honey and control groups decreased. There was no significant difference in this regard between the control group and the Indonesian honey group ($p=0.99$) or the Manuka honey group ($p=0.99$), or between the Indonesian honey group and the Manuka honey group ($p=0.99$).

The numbers of myofibroblasts (Table 1)

On day 3, myofibroblasts in Indonesian and Manuka honey groups were already observed, but no myofibroblasts were observed in the control group. There were significant differences between the control group and the Indonesian honey group ($p=0.012$) and the Manuka honey group ($p=0.007$). However, there was no significant difference between the Indonesian honey group and the Manuka honey group ($p=0.91$).

On day 7, a lot of myofibroblasts appeared in the granulation tissue of the control group and thus the significant differences among all groups disappeared. In this period, the numbers of myofibroblasts of all groups peaked and then decreased gradually on days 11 and 14.

The number of new blood capillaries (Table 1)

On day 3, new blood capillaries in Indonesian and Manuka honey groups were already observed, but no new blood capillaries were observed in wound tissue of the control group. There were significant differences between the control group and the Indonesian honey group ($p=0.0013$) and the Manuka honey group ($p < 0.0001$) in this regard. However, there was no significant difference between the Indonesian honey and the Manuka honey groups ($p=0.26$).

On day 7, the numbers of capillaries of all groups increased and there was no significant difference among all groups. In this period (or on day 11??), the numbers of blood capillaries of all groups peaked and then decreased gradually on days 11(?) and 14.

Rate of re-epithelialization of wound (Table 2)

On day 3 after wounding, the rates of re-epithelialization of Indonesian honey and Manuka honey groups were faster than that of the control group. There was no significant difference between the control group and the Indonesian honey group ($p=0.67$) or the Indonesian honey group and the Manuka honey group ($p=0.14$). However, there was a significant difference between the control group and the Manuka honey group ($p=0.02$).

As the new epithelium of the control group formed rapidly, on day 7, no significant difference of the ratio of re-epithelialization among all groups was observed. On day 11, some wound surfaces were not completely covered with new epithelium, although the wound surfaces of all groups were almost completely covered with new epithelium. On day 14, all wound surfaces were completely covered with new epithelium.

Thickness of wound tissue (Table 3)

On day 3, the thickness of wound tissue for the Indonesian honey group was greater than those of the control group and the Manuka honey group. However, there was no significant difference among all groups. As the wound healing progressed, the thickness of the wound tissue, that is, granulation tissue and scar, of all groups increased. However, there was no significant difference among the three groups on days 7, 11 and 14 after wounding.

DISCUSSION

Indonesia is a developing tropical country that has many kinds of honey. Indonesian honey has been used a lot in topical therapies and traditional medicine for a long time. Therefore, we expected that Indonesian honey could be used for wound care. To date, the effects of Indonesian honey on accelerating wound contraction, re-epithelialization, granulation tissue formation and the distribution of myofibroblasts have not been investigated histologically. Therefore, we investigated the effectiveness of Indonesian honey on wound healing in mice. To the best of our knowledge, this study is the first using Indonesian honey and comparing it with Manuka honey and modern dressing hydrocolloid in mice.

We found by macroscopic observation that the ratios of wound area in the Indonesian honey group on days 2, 5 and 7 were smaller than those of the control group and that there was a significant difference. On day 7, the areas surrounding the wound in Indonesian honey and Manuka honey groups had no redness. Moreover, both groups exhibited clean, newly formed granulation tissue and new epithelium. In a previous study, on day 7 postoperatively, lesions treated with honey in rabbits did not show signs of acute inflammation, and epithelialization occurred.⁸ This result agrees with those in the present report.

By microscopic analysis on day 3, we found that the numbers of neutrophils in Indonesian honey and Manuka honey groups were greater than that of the control group. This was supported by macroscopic findings on days 2 and 5 that redness was observed in the areas surrounding the wounds in Indonesian and Manuka honey groups. However, on day 7, the numbers of neutrophils in Indonesian honey and Manuka honey groups decreased rapidly, and there was no redness in the area surrounding the wounds in both groups, while the control group had edema, and the number of neutrophils in the control group was larger than those in the Indonesian honey and Manuka honey groups. They were significantly different from the

control group. Antibacterial activity of honey could be due to its high osmolarity, low pH (3.6-3.7) and the presence of hydrogen peroxide.^{6,8-9} Hydrogen peroxide forms free radicals¹⁰ that serve to recruit more leukocytes into areas of inflammation, which then promotes the production of pro-inflammatory cytokines by leukocytes.¹¹ Another study has shown that Manuka honey significantly increased the production of pro-inflammatory cytokines.¹² Although the effect of the type of honey on the number of neutrophils by topical administration has not been reported, in a previous study of mice treated by injection with jungle honey from a tropical country, it was shown that the number of neutrophils increased and an effective immune function was observed.¹³ We assumed that honey from Indonesia, a tropical country, may have chemotactic activity on neutrophils. In contrast to our study, another study using pure honey, the type of which was not reported, found that the number of neutrophils was smaller on day 2 than that of a control group.⁸ This difference may be due to the honey properties.¹⁴⁻¹⁵ However, our study showed that inflammation processes with Indonesian honey and Manuka honey were shorter than that of the control group.

Epithelialization is one of the most important factors in wound healing. A previous study in mice showed that the level of wound re-epithelialization was 0.95 mm on day 3, 1.68 mm on day 6 and increased to 2.27 mm on day 9.⁶ Another study reported that acceleration of epithelialization with honey occurred between 6 and 9 days clinically and histologically.⁹ Our findings were that wound re-epithelialization with Indonesia honey reached 26% on day 3, increased to 68% on day 7 and 69% on day 11, and that there was complete epithelialization of the wound surface on day 14. However, the level of wound re-epithelialization with Manuka honey was higher than those of the other groups on day 3 and was significantly different from that of the control group; it increased to 56% on day 7, 86% on day 11 and reached complete epithelialization on day 14. The results for this variable for Indonesian

honey and Manuka honey groups showed no significant differences. On the other hand, on day 3, the re-epithelialization of the control group was about 17% and was slower than that of the experimental group; however, it did rapidly catch up with that of the experimental groups on day 7 and was almost completed on day 11, as in the experimental groups. This indicates that the effectiveness of Indonesian and Monuka honey for re-epithelialization is almost the same as that of modern Tegaderm dressing.

The synthesis of collagen requires energy. This energy may be provided by sugars contained in honey, which enter the glycolysis pathway as a source of energy for fibroblasts and enable synthesis of collagen; this could be shown by the fibroblast proliferation and collagen synthesis on day 8.¹⁶ Another study used histological qualitative assessment that showed that the ratio of thickness of collagen fibers was 60% of normal on day 7, and increased to 85% of normal on day 10.¹⁷ Our results showed that the thickness of wound tissue with Indonesian honey was 0.25 mm on day 3 and reached 0.83 mm on day 14. The results for Manuka honey were 0.22 mm on day 3 and an increase to 0.79 mm on day 14. On the other hand, the results of hydrocolloid dressing were 0.23 mm on day 3 and an increase to 0.83 mm on day 14. These results may indicate that the levels of effectiveness of Indonesian and Manuka honey on the formation of collagen fibers are almost the same as that of hydrocolloid dressing.

The formation of granulation tissue by honey might also occur via the stimulation of growth of fibroblasts by the hydrogen peroxide contained in honey.¹⁸ We found myofibroblasts on day 3 in Indonesian honey and Manuka honey groups. They exhibited significant differences compared with the control group. However, Indonesian honey and Manuka honey groups were not significantly different in this regard. This result showed that the formation of granulation tissue and the proliferation of fibroblasts in Indonesian honey

and Manuka honey groups were faster than those of the control group. A new finding of our study was that, on day 3, new capillaries in the honey groups were observed and they were significantly different from the findings for the control group.

In Indonesia, the prices of Indonesian honey, Manuka honey and Tegaderm hydrocolloid sheet dressing are \$6 per 1000 mL, \$99.99 per ten sheets in a box and \$15 per five sheets in a box, respectively. In the clinical use of Indonesian honey for wound care, 20 mL is used for covering a wound area of 20 cm² for one day, so the amount for 14 days is only 280 mL. Manuka honey involves the use of one sheet per day, so 14 sheets for 14 days are required. The use of hydrocolloid needs one sheet per day with every dressing changed; therefore, during 14 days, 14 sheets are required in total. It can be calculated that the total costs of treatments of Indonesian honey, Manuka honey and Tegaderm hydrocolloid sheet dressing are \$1.68, \$139.98 and \$37.5, respectively, for 14 days of treatment. This shows that Indonesian honey is cheaper than Manuka honey and modern dressing Tegaderm hydrocolloid sheet.

CONCLUSION

The levels of effectiveness of Indonesian honey and Manuka honey in wound healing were almost the same. Indonesian honey and Manuka honey were more effective for depressing inflammation by suppressing the enlargement of the wound area than Tegaderm hydrocolloid sheet dressing. Indonesian and Manuka honey accelerated the formation of granulation tissue more than hydrocolloid dressing. Wound contraction during wound healing by topical treatment of Indonesian and Manuka honey was slow, while that of Tegaderm hydrocolloid sheet dressing was rapid after the wound area enlarged during inflammation. The period before a scar formed in Indonesian and Manuka honey groups was almost the same as that of the hydrocolloid dressing group.

Indonesian honey can be used as a topical therapy as an alternative dressing on various wounds because it is as effective for wound healing as Manuka honey and hydrocolloid dressing, while being cheaper than them.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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Figure legends

Figure 1. Macroscopic observation of wound healing. Day 0 is the day when wounds were made. Note that although wound areas of the control group, the Tegaderm hydrocolloid dressing group, expanded more than those of Indonesian and Manuka honey groups until day 10 after wounding, and wound areas of honey groups decreased step by step, wounds of all groups on day 14 had almost the same area and showed a fine scar. The length between marks is 1 mm.

Figure 2. Ratio of the wound area to the initial area on day 0.

On day 5, the wound areas in the control, Indonesian honey and Manuka honey groups were 1.68 ± 0.12 , 0.59 ± 0.02 and 0.78 ± 0.01 times as large as on day 0, respectively, and there were significant differences between the control group and the Indonesia honey group ($p=0.0001$) and the Manuka honey group ($p=0.0001$).

On day 14, the wound areas in control, Indonesian and Manuka honey groups were 0.30 ± 0.08 , 0.31 ± 0.14 and 0.22 ± 0.04 , respectively. There were no significant differences between the control group and the Indonesian honey group ($p=0.98$) and the Manuka honey group ($p=0.35$).

Figure 3. Histology on 3 and 7 days after wounding stained with H and E, Azan method and anti- α SMA antibody.

Control (hydrocolloid dressing) group on day 3 (A-1~A-3) and on day 7 (A-4~A-6).

Indonesian honey group on day 3 (B-1~B3) and on day 7 (B-4~B6), and Manuka honey group on day 3 (C-1~C3) and on day 7 (C-4~C-6).

A lot of neutrophils (arrows) were observed in wound areas of all groups on day 3 and decreased slightly on day 7; edges of new epithelium (arrowheads) were already present at the wound edge on day 3 (A-1, A-4, B-1, B-4, C-1, C-4).

Thin collagen fibers stained dark blue were already present across the wound area in all groups on day 3 and became bundles of collagen and made up a part of the granulation tissue on day 7 (A-2, A-5, B-2, B-5, C-2, C-5).

Although non-specific staining was present in the wound tissue of the control group on day 3 (A-3), neither myofibroblasts nor new blood vessels could be found. On the other hand, myofibroblasts (arrows in B-3, C-3) and new blood vessels (arrowheads in B-3, C-3) were observed. On day 7, a lot of myofibroblasts (arrows in A-6, B-6, C-6) and new blood capillaries (arrowheads in A-6, B-6, C-6) were present in the wound area of all groups, which were related to the granulation tissue.

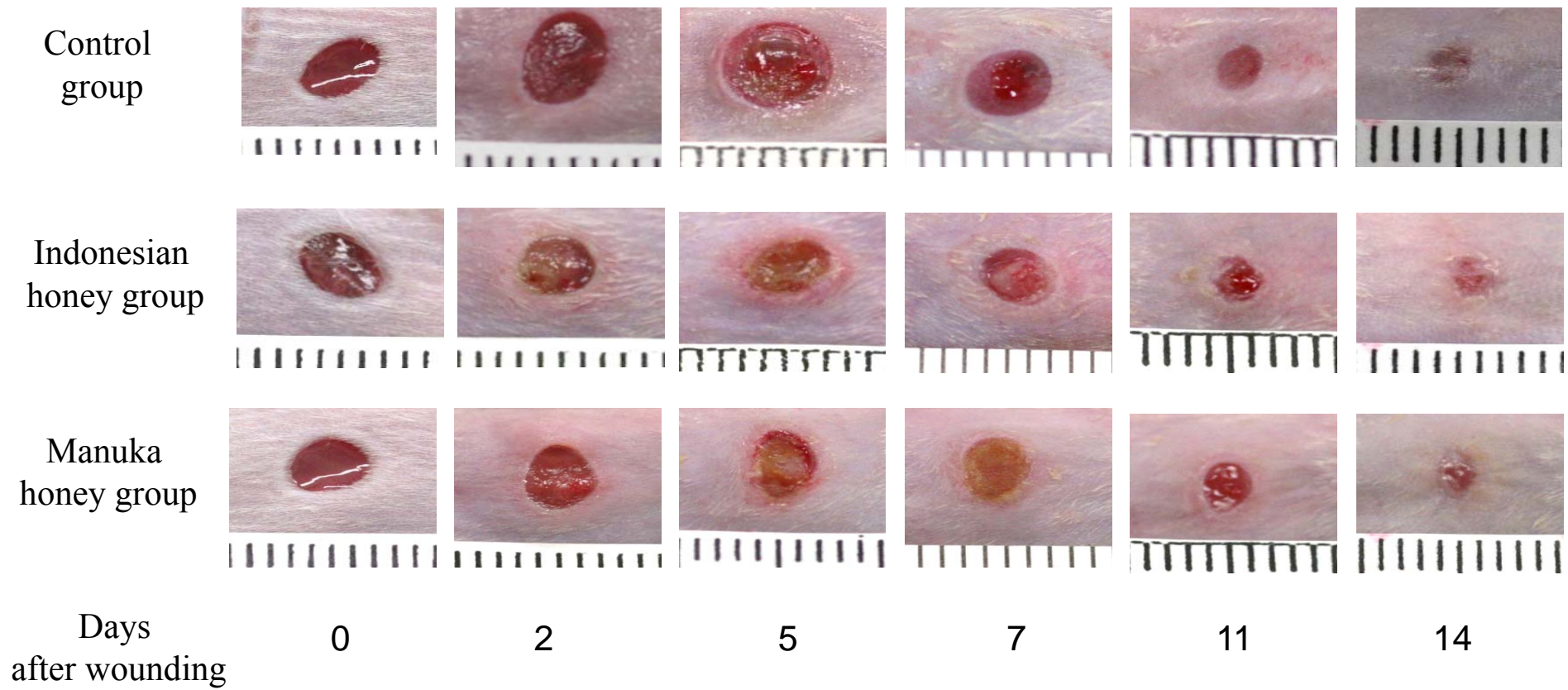
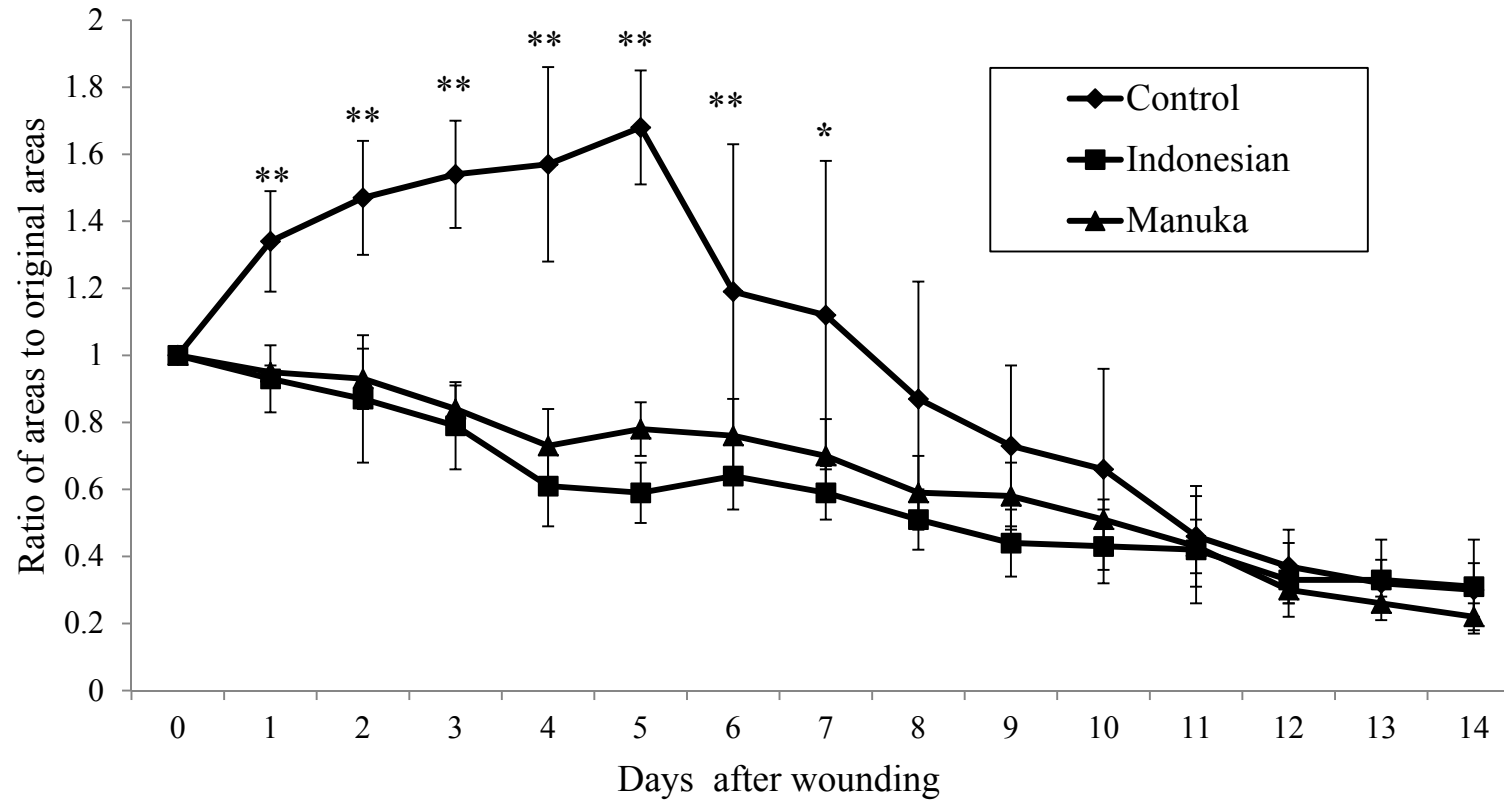


Figure 1. Macroscopic observation of wound healing



Value was expressed Mean \pm SD. n = 6 per group . ANOVA, Tukey-Kramer
 (* $p < 0.05$, ** $p < 0.01$)

Figure 2. Ratio of the wound area to initial area on day 0

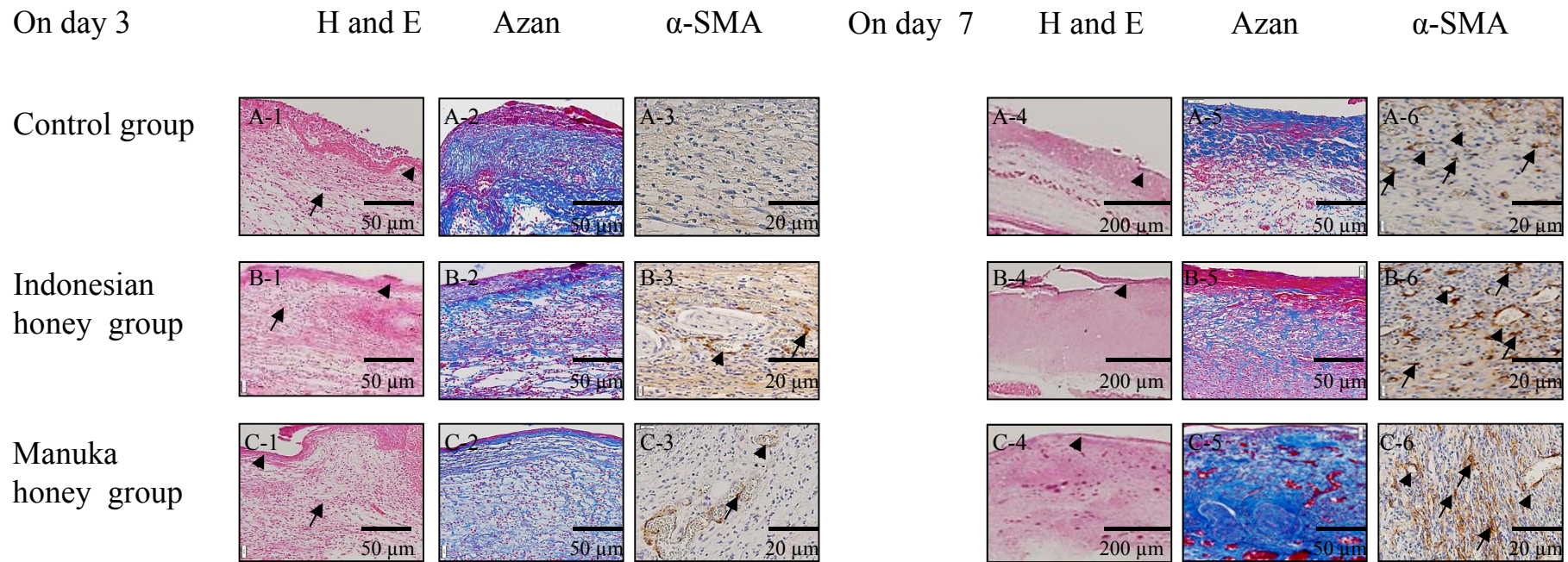


Figure 3. Histology on 3 and 7 days after wounding stained H and E, Azan Method, and α -SMA

Table 1. Number of neutrophils, myofibroblasts and new blood capillaries (/mm²)

The Number of neutrophils

Groups	Days after wounding			
	3	7	11	14
Control	2268.00 ± 364.96	1157.83 ± 88.50	203.50 ± 42.50	129.20 ± 25.37
Indonesian	3336.67 ± 254.39	1579.60 ± 407.69	202.50 ± 30.25	140.66 ± 29.53
Manuka	3177.57 ± 383.84	1169.67 ± 51.24	201.80 ± 29.65	108.50 ± 4.23

Control=5, Indonesian=6, Manuka=6

The number of myofibroblasts

Groups	Days after wounding			
	3	7	11	14
Control	00.00 ± 00.00	195.16 ± 54.48	138.16 ± 25.03	127.66 ± 20.76
Indonesian	47.80 ± 27.08	210.50 ± 68.31	139.00 ± 25.72	129.00 ± 10.65
Manuka	42.33 ± 27.60	232.00 ± 37.74	152.60 ± 51.40	106.33 ± 48.42

Control=6, Indonesian=6, Manuka=5

The number of new blood capillaries

Groups	Days after wounding			
	3	7	11	14
Control	00.00 ± 0.00	39.00 ± 5.21	43.25 ± 21.26	21.25 ± 8.13
Indonesian	12.33 ± 5.00	43.00 ± 6.32	46.25 ± 26.51	16.50 ± 12.44
Manuka	16.83 ± 6.61	42.00 ± 7.79	50.40 ± 11.39	18.00 ± 6.21

Control=6, Indonesian=6, Manuka=6

Value are expressed as mean ± SD, ANOVA, Tukey-Kramer **p* < 0.05 ***p* < 0.01

Table 2. Rate of re-epithelialization of wounds (%)

Groups	Days after wounding			
	3	7	11	14
Control	17 ± 14	59 ± 17	69 ± 41	100 ± 00
Indonesian	26 ± 11	68 ± 16	84 ± 24	100 ± 00
Manuka	48 ± 27	56 ± 14	86 ± 13	100 ± 00

Control=5, Indonesian=5, Manuka=5

Value are expressed as mean ± SD, ANOVA, Tukey-Kramer * $p < 0.05$

Table 3. Thickness of wound tissue (mm)

Groups	Days after wounding			
	3	7	11	14
Control	0.23±0.22	0.38±0.13	0.65±0.11	0.83±0.23
Indonesian	0.25±0.02	0.47±0.09	0.61±0.19	0.83±0.36
Manuka	0.22±0.21	0.51±0.13	0.59±0.22	0.79±0.22

Control=5, Indonesian=5, Manuka=5

Value are expressed as mean±SD, ANOVA, Tukey-Kramer