

COMPARISON ON INFECTED WOUND HEALING TIME, USING COFFEE POWDER AND HONEY IN WISTAR RAT

YOHANES SETYAWAN¹, HENDRO SUDJONO YUWONO² & RUDOLF ANDEAN³

¹Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia

²Department of Surgery, Faculty of Medicine, Universitas Padjadjaran/ Hasan Sadikin General Hospital,
Bandung, Indonesia

³Department of Physiology, Faculty of Medicine, Universitas Padjadjaran/ Hasan Sadikin General
Hospital, Bandung, Indonesia

ABSTRACT

Background: Wound infections are the most possible complication, if a wound is not treated properly. The most common microorganism that causes wound infections is, *Staphylococcus aureus*. Antimicrobial resistance is rising rapidly, but not balanced with the development of antibiotics, therefore an alternative choice for the treatment of wound infections is required. Coffee and honey have been studied and proved to have the ability, to quicken wound healing, but no study has been done to compare these two. This study aimed to discover the differences of infected wound healing using coffee powder and honey.

Methods: This experimental study was using 20 male Wistar rats as subjects, which were divided into four groups according to the treatment of wound infections (coffee powder, honey, positive control, and negative control). Every week within four weeks, assessment of infected wound healing was done according to three macroscopic indicators (wound was dry; the edge of the wound was not hyperemic, and normal leukocyte count). The result of this study was infected wound healing time in weeks and analyzed using survival analysis method.

Results: Mean of wound healing time in coffee powder group (2 weeks) was quicker than honey group (3.4 weeks) and statistically significant with p-value=0.03.

Conclusions: This study discover that there is a significant differences on infected wound healing using coffee powder compared with honey. Infected wound healing time using coffee powder is quicker than honey. Further study is required to find out the effective doses of coffee powder and honey.

KEYWORDS: Coffee Powder, Honey, Infected Wound Healing Time, Wistar Rats, Wound Infection

INTRODUCTION

Wound is a damage that is accompanied by disruption of normal continuity of a structure.¹ When a wound occurs, the body would respond by carrying out wound repair. This process needs to be supported by proper wound management. Inappropriate wound management can result in wounds that do not heal, which indicates a neglected bacterial infection.² Silver sulfadiazine is the gold standard for wound management; it works by inhibiting bacterial growth and thus decreasing the risk of bacterial spreading into the blood stream (sepsis).³

The most commonly found infection-causing bacteria in wounds is *Staphylococcus aureus* (SA). In 2014, WHO reported that SA was becoming more resistant towards antibiotics in several countries.⁴ This resistance could lower the effectiveness of antimicrobial agents in the treating infected wounds. Although various drugs can be used to treat infected wounds, only a few is without limitations.² One alternative available to us is to use natural substances to treat bacterial infections in wounds. Coffee and honey are two examples of natural substances whose ability to prevent MRSA infection and hasten wound healing has been proven.

Robusta coffee powder has chlorogenic acid, which is strongly suspected of having antioxidant effect and antibacterial effect against Methicillin-Resistant *Staphylococcus aureus* (MRSA).⁵ Its ability in speeding up wound healing has been extensively studied and confirmed that, it can hasten healing of wounds on the body surface.^{5,6}

Honey is an ancient remedy for management of wound infections and was first used as a topical antibacterial agent in 1982.⁷ Honey, especially of the Manuka variety, contains methylglyoxal (MGO), which has an important role in speeding up wound healing and preventing infection in wounds.

These two substances have the ability to speed up wound healing but no study has compared them both. This study aimed to discover the differences of infected wound healing using coffee powder and honey.

METHODS

This is an experimental study with post-test only controlled group design. This study takes place in Pharmacology and Therapy Laboratory of Faculty of Medicine Universitas Padjadjaran Bandung, from September to November 2015. The methods have fulfilled the ethical requirements for laboratory animals welfare principle.⁸ Ethical clearance letter had also granted by the Health Research Ethics Committee Faculty, of Medicine Universitas Padjadjaran Bandung No.531/UN6.C1.3.2//KEPK/PN/2015.

The subjects are male Wistar rats (*Rattus norvegicus*), with a minimum sample size of 20, as calculated using Mead's resource equation.⁹ The inclusion criteria for the subjects are male sex, the weight of 250-300 gram, the age of 8-12 weeks, and good health, as shown by clean fur, active movement, and absence of wounds. The subjects are excluded when they are sick, suffer from >10% weight loss during or after adaptation, have previously participated in other experiments, or die during adaptation or the experiment itself.

All 20 rats went through an adaptation period of 7 days in the laboratory. Each rat lived in one cage separated by wire net to prevent other rats from licking the wounds and fighting between rats, but they could still express their natural social habit. After the adaptation period, hair on the rats' back was shaved, and an incision wound 3x3 cm and 2 mm thick (until the superficial fascia was seen) was made. Then 1 ml bacterial suspension of American Type Culture Collection-25923 *Staphylococcus aureus*, with McFarland density of 0.5 (10^8 CFU/mL), obtained from Microbiology Laboratory of Faculty of Medicine Universitas Padjadjaran Bandung, was injected intracutaneously. After the injection, the wound was left alone for 4 days to encourage infection. The 20 rats divided into 4 groups of 5 rats. Experimental treatments were started on the fourth day, after the bacterial injection.

The treatments were given once a week for 4 weeks. There were 4 treatments, each one for a different group: Robusta coffee powder group, Manuka honey group, positive control group, and negative control group. Robusta coffee powder was obtained from a local market (Aroma coffee shop, Bandung, Indonesia) whereas the Manuka honey obtained

from a department store in Bandung, Indonesia. The rats in the coffee powder and honey groups had their wounds thoroughly coated until the edges, with Robusta coffee powder and Manuka MGO30+ honey respectively, and then covered with sterile dressing. Rats in the positive control group had their wounds treated with silver sulfadiazine cream and covered with a sterile dressing. Rats in the negative control group only had their wounds covered with a sterile dressing without any treatment. Every one week, the coffee powder, honey, silver sulfadiazine cream, and the dressing were replaced. Wound drying measured during the replacement.

The infected wounds were said to be dry and starting to heal if they fulfilled the following 3 indicators: wound was dry, the edge of the wound was not hyperemic, and normal leukocyte count ($6-17 \times 10^3/\text{mm}^3$). The first two indicators were done by observation, while the leukocyte counted using Neubauer chamber (hemocytometer). The week in which all 3 indicators were first met, recorded for each subject. The indicators were examined weekly for 4 weeks. After 4 weeks, the data was analyzed using survival analysis with healing as the event.

RESULTS AND DISCUSSIONS

The first indicator of infected wound healing in this study is the wound was dry. Drying of wounds was found in all coffee powder, positive control, and negative control groups’ members in the second week. In the honey group, all the wounds only dried in the fourth week, which might be due to the wetness of honey that prevented the absorption of wound fluid. Drying of wound surface is an initial sign of good wound healing since it shows that fluid production (oozing) from the wound has stopped as re-epithelialization of the wound surface completes.⁶ The collected data showed that coffee powder proved to be more efficient in drying wound surface compared to honey. This was caused by the hygroscopic property of coffee powder which makes it better in absorbing fluid from the wound compared to honey.⁶ (Figure 1)

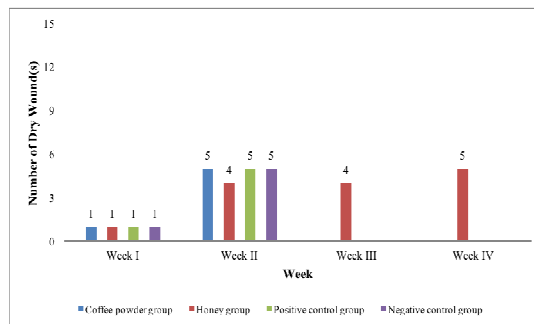


Figure 1: The Pattern of the Frequency of Wound Drying among the Four Groups

Hyperemic wound edge is a sign of inflammation, which can be found in infected wounds.¹⁰ Inflammation occurs as an effect of pro-inflammatory cytokines, such as IL-1 and TNF- α . Bacteria and endotoxin can cause prolonged elevated levels of the two cytokines, resulting in prolonged inflammation phase.¹¹ The disappearance of hyperemia, along the wound edge marks a wound healing process. The second indicator of infected wound healing in this study is when the wound edge was no longer hyperemic. In the first week, there were 4 rats with non-hyperemic wound edges in the coffee powder group, different from the other 3 groups. All rats in the coffee, positive control, and negative control groups had no more hyperemic wound edges by the third week while the rats in the honey group were free of hyperemia in the fourth week. This study discovered that the disappearance of hyperemia along the wound edge occurred earlier in the coffee, positive control, and negative control groups than in the honey group. As such it could be concluded that coffee powder was

better than honey in lowering the excessive levels of pro-inflammatory cytokines produced in response to bacterial infection. (Figure 2)

One microscopic indicator of bacterial infection is elevated blood leukocyte count.² In this study, the third indicator of infected wound healing is the leukocyte count is within the standard limit. The coffee group had its leukocyte examined only up to the third week because the leukocyte count of the subjects in that group had all reached normal range by the third week. In the first week, the lowest mean of leukocyte count was found in the positive control group, which was $13.30 \times 10^3/\text{mm}^3$. In the second and third week, the lowest mean of leukocyte count was found in the coffee group: $13.60 \times 10^3/\text{mm}^3$ and $14.98 \times 10^3/\text{mm}^3$ respectively. Coffee powder was faster than honey in returning leukocyte count into the normal range. Hence it could be concluded that the coffee powder had a better antibacterial effect than honey. (Figure 3)

Infected wound healing time data, the week in which all 3 indicators were first met, was analyzed using survival analysis method. A survival function graph presents when the event occurs. In this study, the event was the drying of infected wound, which was represented as a straight line. Circles represent that the data was censored or the event had not happened yet. All of infected wounds in the coffee group were dry by the third week. In the honey and positive control group, 1 out of 5 members still had undried wound in the fourth week. In the negative control group, 2 infected wounds were dried in the fourth week, while the other 3 were not. (Figure 4)

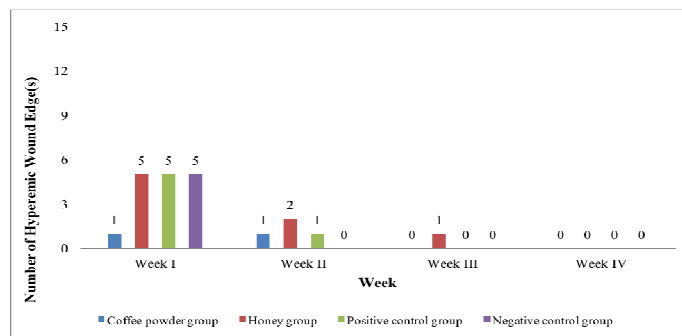
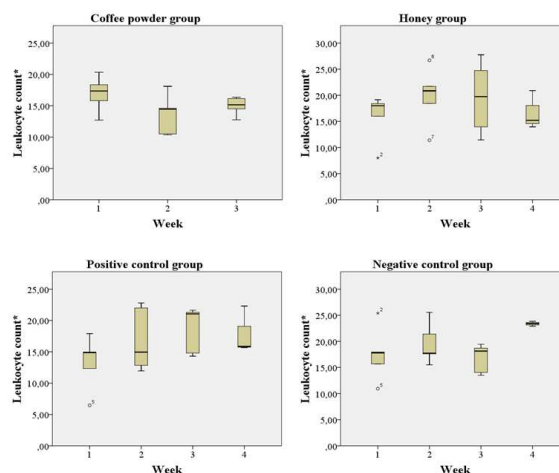


Figure 2: The Pattern of Frequency Hyperemic Wound Edge among the Four Groups



*Leukocyte count in ($\times 10^3/\text{mm}^3$)

Figure 3: The Pattern of Leukocyte Count among the Four Groups

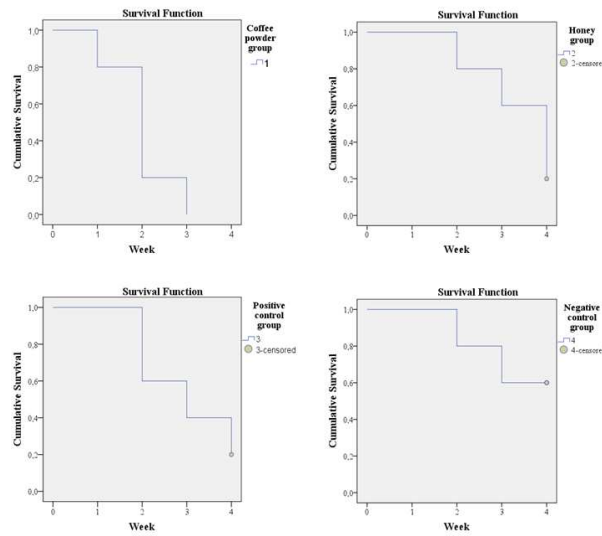


Figure 4: Survival Function with Event (Infected Wound Healing)

The data of the four groups were distributed as $p > 0.05$. The drying time of infected wound was quicker in the coffee powder group compared to the other three groups. The drying time of infected wound in the honey group was the same with the negative control group. (Table 1)

Table 1: Mean of Drying Time of Infected Wounds among the Four Groups

Group	Mean (Weeks)
Coffee Powder	2.00
Honey	3.40
Positive Control	3.00
Negative Control	3.40
Total	2.95

The log rank analysis produced a p-value of 0.036. As it was lower than 0.05, it could be concluded that the results gathered from survival analysis were significant. (Table 2)

Table 2: Hypothesis Testing on Comparison of Inter-Group Survival Distribution

	Chi-Square	p-Value
Log Rank (Mantel-Cox)	8.560	0.036

The author recommends further studies on the necessary dosage of coffee powder and honey as this study did not measure the amount of the treatments. This study used Manuka honey that has MGO30+, which may have a significant effect on the results. Besides MGO30+, there is Manuka honey with higher levels of MGO. However, the effect of MGO levels in Manuka honey, to the rate of healing of infected wounds has not studied yet.

CONCLUSIONS

There was a difference in drying time of infected wound, using coffee powder and honey. The use of Robusta coffee powder caused infected wounds, to dry earlier than the wounds treated with Manuka honey. The mean drying time of the honey group was equal to the one of the negative Control group.

Disclosure. The authors have no conflicts of interest to disclose.

Acknowledgement. Not any funding support to this study.

REFERENCES

1. Dorland WAN. Dorland's Illustrated Medical Dictionary³²: Dorland's Illustrated Medical Dictionary: Elsevier Health Sciences; 2011.
2. Gottrup F, Apelqvist J, Bjarnsholt T, Bjansholt T, Cooper R, Moore Z, et al. EWMA document: Antimicrobials and non-healing wounds. Evidence, controversies and suggestions. *Journal of wound care*. 2013; 22(5 Suppl):S1-S92.
3. Majtan J. Methylglyoxal—a potential risk factor of manuka honey in healing of diabetic ulcers. *Evidence-based Complementary and Alternative Medicine*. 2010;2011:5.
4. WHO. Antimicrobial resistance: global report on surveillance. World Health Organization; 2014. p. 257.
5. Susanto Y, Puradisastra S, Ivone J. Effect of Robusta coffee bean powder (*Coffea Robusta* Lindl.de Wild) on time of wound closure on male strain Balb / C-induced mice. *Maranatha Medical Journal*. 2010; 8(2):pp. 121-6.
6. Yuwono HS. The New Paradigm of Wound Management Using Coffee Powder. *Journal of Surgery*. 2014; 2(2): 25-9.
7. Winata Aa, Yuwono HS & Hapsari P, Correlation between MMP-1 Responses and Epithelialization of Acute Staphylococcus Aureus Infected Wounds Treated by Coffee Powder, Saline Gauze, and Hydrocolloid, *International Journal of General Medicine and Pharmacy (IJGMP)*, Volume 6, Issue 5, August-September 2017, pp. 31-38
8. Grothier L, Cooper R. Medihoney™ Dressings Made Easy-Products for Practice. *Wounds-UK*. 2011; 6(2):4.
9. Ridwan E. Ethical Utilization of Animal Experiments in Health Research. *Journal of the Indonesian Medical Association*. 2013;63(3):112-6.
10. Festing MF. How to reduce the number of animals used in research by improving experimental design and statistics. *ANZCCART Fact Sheet*. 2011; 10:1-11.
11. Torpy JM, Burke A, Glass RM. Wound infections. *JAMA*. 2005; 294(16):2122.
12. Guo S, DiPietro LA. Factors affecting wound healing. *Journal of dental research*. 2010; 89(3):219-29.