

pH Of Wound Fluids Treated Using Coffee Powder and Bacitracin-Neomycin Powder

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Abstract Introduction: Coffee powder has been known for its traditional remedy for wounds because of its effectiveness in wound healing. The process of wound healing is influenced by various factors, including the wound-pH. The pH of coffee powder is 4.5-5, which is also the optimal pH for wound healing. The following study will compare the wound-pH of coffee powder with Bacitracin-Neomycin powder (BN). **Methods:** Wistar rats were divided into three groups, namely coffee group (group I), BN group (group II) and control group (group III), in which the pH measurements were taken at the 24th-hour and 7th-day of treatment respectively. Data analysis was done as the Shapiro Wilk test or Kruskal Wallis test depending on parametric or non-parametric data, and the significance was rated using One Way Anova. **Results:** At the 24th-hour of treatment the wound-pH of group I was significantly lower compared to group II ($p=0.010$) and group III ($p=0.002$). At the 7th- day of treatment, group I was unsignificantly lower than group II ($p=0.105$), but was significantly lower than group III ($p = 0.003$), where as group II was unsignificantly lower than group III ($p=0.323$). **Conclusion:** This study showed that the pH of the wound fluid which used coffee powder is lower than the BN powder.

Keywords: pH, coffee powder, Bacitracin-Neomycin

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1. Introduction

Wound healing is a complex regenerating process by distinct phases of inflammation, proliferation, and remodeling, in which all phases are affected by various factors, including pH of wound fluids. Wound-pH influences the biochemical reactions of wound healing process, such as an increase in the effects of protease activity and the release of oxygen, reduces the toxicity of bacteria, triggers angiogenesis, as well as increases the activity of macrophages and fibroblasts [1,2]. Wound surface pH depends on the techniques and materials used in wound care. The ideal topical wound dressing does not only accelerate wound healing, but also able to reduce the loss of protein, electrolytes and fluids from the wound, as well as helps to relieve pain [3].

The treatment of wounds using coffee powder have started since hundred-years ago as a folk remedy for its effectiveness in healing wounds. Many health providers at coffee-plantation areas reported that the use of coffee powder on every wounds, either acute or chronic (diabetes mellitus, sharp cuts, burns) are left without complication [4].

Coffee powder has a natural pH of 4.5-5, almost accordance to the skin pH of 4.5-6. Bacterial growth in wound exudate caused by inflammation or infection has pH of 7.3. While wound-pH of 4-6 creates an unfavorable environment for bacterial growth, it will reduce the chance of inflammatory complication [5].

Topical Bacitracin-Neomycin powder is a common antibiotic used in clinical practice for a variety of uses, including topical antibiotic for post-operative surgical wounds and as a prophylaxis regiment against infection. Bacitracin-Neomycin powder and as an antibiotic combination also makes the wound more acidic and prevent microbial growth. [6].

The aim of this study was to compare the pH of wounds treated topically using coffee powder and Bacitracin-Neomycin powder.

2. Materials and Method

2.1. Research Design

This is an experimental research to determine the differences in wound-pH and was approved by the institution's ethical committee.

2.2. Inclusion Criteria

Male Wistar rats, aged 2-3 months, weighed 200-300 grams, healthy and active, with no sign of infection, hairy slick, shiny and clean, it has been quarantined for one week, and free from outside influences.

2.3. Exclusion Criteria

Unhealthy rats during the quarantine period or during study, and rats weighed <200 g or > 300gr before the study being conducted.

2.4. Sample Size

The sample size of each group was determined using Federer formula:

$$(n - 1) (t - 1) \geq 15, \text{ resulted } n \geq 8,5$$

t : the number of group.

n : sample size.

In this study 9 animals were used as a minimum sample size for each group.

2.5. Powder Used

Nebacetin powder (bacitracin 250 IU, neomycin sulfate 5 mg) from Pharos Indonesia was used in this experiment. A new freshly made Coffee arabica powder was obtained from a local market, i.e. Aroma coffee shop in Bandung, Indonesia. The pH of Neomycin is 6.8; Bacitracin 1% solution in water has a pH of 6.0 to 7.0 [6,7].

2.6. Animal Procedures

Each rat was placed in a separate cage, at room temperature with normal lighting and was fed with pallet and adequate water.

The wounds were made after the rats were anesthetized using intramuscular ketamine 0.4 ml injecting into the rat's thigh. Then, 70% alcohol solution swab were applied on the back of the rats, which were than shaved and made wounds. measuring 4 cm x 4 cm (rectangular), with a depth of 2 mm into the subcutaneous fat layer.

Then, the wistar rats were divided into three groups, namely coffee group (group I), BN group (group II) and Control group (group III), in which each group was measured for the pH of the wound which was taken at the 24th-hour and 7th-day of treatment.

2.7. Statistical Analysis

After collecting data, all variables were analyzed using Shapiro Wilk test for parametric data and Kruskal Wallis test for non-parametric data.

All groups were statistically analyzed using SPSS version 19 for Windows, with p smaller than 0.05 was considered significant, and post-HOC test with Dunn test was applied.

3. Results

The mean of wound-pH at 24th-hour treatment with coffee powder was 5.222, and the median was 5, in which the lowest was 4 and the highest pH was 6. The mean wound-pH for the BN treatment was 6.333, and the median was 6, with the lowest pH of 5 and the highest pH of 7. The mean wound-pH of the control group was 6.556, and the median was 7, with the lowest pH of 6 and the highest pH of 7.

At the 7th-day of treatment, the mean wound-pH of coffee group was 5.889, the median was 6, the smallest was 5 and the highest was 7. The mean wound-ph of BN category was 6.667, the median was 7, the lowest is 6 and the highest was 8. While the mean in the Control category was 7.111, the median was 7, the lowest was 6 and the highest was 8.

In Table 1, there was a significant difference (p <0.001) of wound-pH at the 24th -hour and at the 7th -day of treatment (p=0.010) of the coffee (Group I), BN (Group II), and Control (Group III).

Table 1. Kruskal-Wallis Test

Output	Category			Total (n=27)	p Value
	Coffee (n= 9)	BN (n=9)	Control (n = 9)		
pH 24 th -hour median,(min; maks)	5(4;6)	6(5;7)	7 (6; 7)	6(4;7)	<0,001 ^s
pH after the 7 th -day median, (min; maks)	5(4;6)	6(5;7)	7 (6; 7)	7(5;8)	0,010 ^s

Min=minimum; \$=significant; Coffee=Group I, BN=Group II, Control=Group III.

Table 2. Post Hoc test with Dunn test at the 24th-hour treatment

Comparison	Difference median (trust interval) [#]	p Value ^s
Coffee vs BN	-1 (-2; -0)	0,010
Coffee vs Control	-1 (-2; -1)	0,002
BN vs Control	-3 (-1; 1)	0,839

Based on Table 2 the wound-pH of Group I was significantly lower (p=0.010) than Group II and Group III (p=0.002) at the 24th-hour treatment.

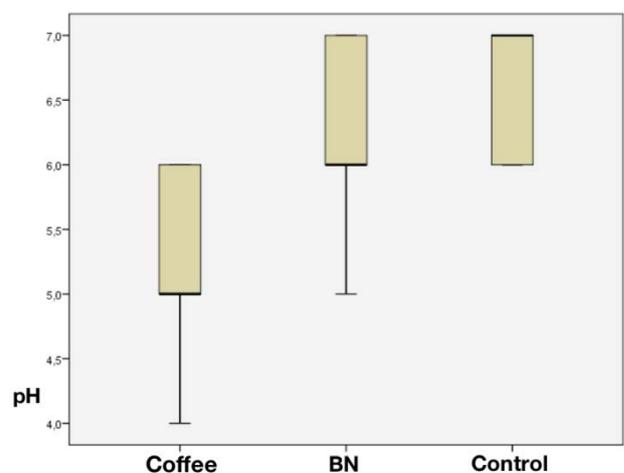


Figure 1. The wound-pH of Coffee Group in comparison with BN and Control Groups at 24th-hour treatment

Table 3. Post Hoc test with Dunn test at 7th-day treatment

Comparison	Difference median (trust interval) [#]	p value ^s
Coffee vs BN	-1 (-2; 0)	0,105
Coffee vs Control	-1 (-2;-0)	0,003
BN vs Control	-5 (-1; 0)	0,323

Table 3 showing insignificant difference (p=0.105) between the Group I and Group II at the 7th-day treatment. At the 24th- hour post-treatment, wound-pH of Group I was significantly lower (p = 0.003) than Group III.

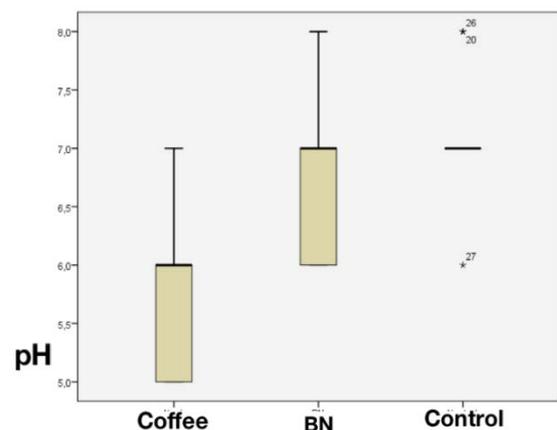


Figure 2. The pH at 7th-day treatment of Coffee group in comparison with BN and Control

4. Discussion

4.1. Acidity as Wound Infection Control

Under normal circumstances, the pH value that found on the skin surface of human body described by Hesus et al, Schade and Marchionini is 4-6 and is an important aspect of skin protection against bacteria growth. The pH value of the skin is also an important thing against harmful chemicals from the environment. The normal pH value of the skin derived from amino acids, fatty acids and other products that serve as a buffer, such as organelles and keratin layer of the skin [8].

An attempt to decrease wound surface pH using topical acid agents, such as citric acid for chronic wound, dilute acetic acid (1% and 5% concentrations) for burn wound infections, has been used in an effort to lower the pH, have had varying degrees of efficacy [9,10].

The insignificant difference of wound-pH between Coffee and BN at the 7th-day of treatment can occur because of the effectivity of BN to reduce the activity of proteolytic bacteria, thus making the wound-pH more acidic and hence preventing the microbial colonization [1,6].

4.2. The Role of Acidity in Wound Healing

Results of this study confirmed the literature that coffee beans contain a pH of 4.5-5, in accordance to the skin pH of 4,5-6 and correspond well with the optimum pH for wound healing. In the process of wound healing, the pH of the surface of wound was initially neutral in line with the wound healing process, increased protease and release of oxygen affect the pH levels on the surface of the wound will be the acid, thereby reducing the toxicity of the bacteria and their products (ammonia) increase the destruction of collagen in the wound, triggering angiogenesis, increasing the activity of macrophages and fibroblasts and enzyme activity. Bacterial growth will result in inflammation or infection with a pH of 7.3 in wound exudate. While the low pH in the wound fluid creates an unfavorable environment for bacterial growth. [2,5] An acidic environment in the wound would create something that is very favorable in terms of antimicrobial activity, infection control, release of oxygen, promotes epithelization and angiogenesis [9,10,11]. Acidity is also very important to keep the first phase of wound healing (inflammatory phase) run optimally, thus preventing the elongation of time of this phase, which can be caused mainly by the growth of microbial contamination. Almost all of the pathogenic bacteria correlated with the infected wounds in human need pH more than 6, their growth is suppressed by lower pH values [12]. The results showed that wounds treated with coffee and BN were having a pH ≤ 6 i.e. low enough to prevent the growth of pathogenic bacteria, thereby accelerating healing process.

4.3. Study Limitations

This study is limited by the measurement of wound-pH using a pH meter that was difficult to be done due to limited wound fluids, which were only be measured by using pH paper test (litmus paper). Measurement using litmus paper has its limitation, where the result is not as accurate compared to using a pH meter. This study has

limitations, namely the use of mice as research objects that have multiple barriers, such as fixation of wound dressing which was sometimes open. So that researchers must often control and make improvements mounting the fixation plaster gauze. Moreover, wound fluid which partly absorbed in gauze dressings also affects the measurement of pH.

5. Conclusion

It can be concluded that the wound-pH in Coffee group was lower compared to BN after 24th-hour of treatment, but there was no significant difference in wound-pH between Coffee group dan BN at 7th-day of treatment.

Author's Contribution

Delidios designed the study and the statistical analysis, wrote the protocol, and wrote the first draft. H.S. Yuwono analyzed the study and the references. Both authors read the last version and approved the manuscript.

Conflict of Interest

The authors confirm that this article has no conflict of interest.

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