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EFFECTIVENESS OF NaCl 0.9% WITH GARAMYCIN AND ALOE VERA ON DIABETIC WOUND HEALING IN RATS

Efektivitas NaCl 0,9% dengan Garamycin dan Aloe Vera terhadap Penyembuhan Luka Akut Diabetes pada Tikus

Sri Mulyati Rahayu[1*], Ali Hamzah[2], Vina Vitniawati[1]
[1]Program Studi D III keperawatan, Fakultas Keperawatan, Universitas Bhakti Kencana, Bandung, Indonesia
[2]Program Studi Sarjana Terapan Keperawatan, Jurusan Keperawatan, Poltekkes Kemenkes Bandung, Bandung, Indonesia
*Email: sri.mulyati@bku.ac.id

ABSTRAK

*Ulkus diabetik dimulai sebagai luka biasa, tetapi tanpa penanganan yang tepat berisiko menjadi infeksi, ulserasi, gangren, dan amputasi. Luka yang terkontaminasi meningkatkan pertumbuhan dan kolonisasi mikroba. Penelitian ini bertujuan menentukan efektivitas kompres NaCl 0,9% dengan gel aloe vera dibandingkan dengan NaCl 0,9% dengan garamicyn dalam mengobati ulkus diabetik akut pada tikus. Metode penelitian dengan eksperimental, menggunakan 16 tikus ulkus diabetik yang diobati dengan prinsip pembalut lembab. Jumlah koloni bakteri dinilai sebelum dan sesudah tujuh hari perawatan untuk mengevaluasi efektivitas pembalut lembab yang ditambah dengan aloe vera atau garamisin. Kebaruan penelitian ini terletak pada perbandingan prinsip pembalut lembab NaCl 0,9% dengan garamycin atau aloe vera gel pada ulkus diabetik tikus. Hasil analisis Wilcoxon menghasilkan nilai-p 0,012 (<0,05), yang menunjukkan pengurangan signifikan pada koloni bakteri dengan NaCl 0,9% ditambah aloe vera (*146,875*) dengan daya hambat sedang. Analisis uji-t berpasangan menunjukkan nilai p 0,01 (<0,05), yang menunjukkan penurunan signifikan antara NaCl 0,9% dan garamysin (*221,875*) dengan daya hambat kecil. Nilai signifikansi yang lebih kecil pada uji-t berpasangan menunjukkan bahwa NaCl 0,9% dengan garamysin lebih efektif daripada NaCl 0,9% dengan aloe vera. Kesimpulannya, terdapat perbedaan yang signifikan secara statistik antara jumlah koloni bakteri sebelum dan sesudah perawatan ulkus diabetik dengan NaCl 0,9% dan aloe vera serta NaCl 0,9% dan garamicyn. Perawatan ulkus diabetik pada tikus dengan NaCl 0,9% dan garamisin lebih efektif (76,08%) daripada NaCl 0,9% dan aloe vera (57,54%). Rekomendasi perlu penelitian lebih lanjut pada manusia dengan ulkus diabetik akut.*

***Kata kunci**: aloe vera, diabetikum, garamycin, luka, NaCl 0.9%*

ABSTRACT

Diabetic ulcers begin as ordinary wounds, but without proper treatment, they are at risk of infection, ulceration, gangrene, and amputation. Contaminated wounds increase microbial growth and colonization. This study aims to determine the effectiveness of 0.9% NaCl compresses with aloe vera gel compared to 0.9% NaCl with garamycin in treating acute diabetic ulcers in rats. The experimental research method used 16 diabetic ulcer rats treated with the principle of moist dressings. The number of bacterial colonies was assessed before and after seven days of treatment to evaluate the effectiveness of moist dressings supplemented with aloe vera or garamycin. The novelty of this study lies in the comparison of the principle of moist dressings of 0.9% NaCl with garamycin or aloe vera gel on diabetic ulcers in rats. The results of the Wilcoxon analysis produced a p-value of 0.012 (<0.05), which indicates a significant reduction in bacterial colonies with 0.9% NaCl plus aloe vera (146,875) with moderate inhibition. Paired t-test analysis showed a p value of 0.01 (<0.05), which indicated a significant decrease between 0.9% NaCl and garamycin (221.875) with a small inhibitory power. A smaller significance value in the paired t-test indicated that 0.9% NaCl with garamycin was more effective than

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0.9% NaCl with aloe vera. In conclusion, there was a statistically significant difference between the number of bacterial colonies before and after treatment of diabetic ulcers with 0.9% NaCl and aloe vera, and 0.9% NaCl and garamycin. Treatment of diabetic ulcers in rats with 0.9% NaCl and garamycin was more effective (76.08%) than 0.9% NaCl and aloe vera (57.54%). Recommendations require further research in humans with acute diabetic ulcers.
Keywords: *Aloe vera, Diabeticum, Garamycin, NaCl 0.9%, Wound*

INTRODUCTION

Acute wounds are wounds that occur suddenly. Wound condition assessment and wound care management greatly influence the wound healing process. Knowledge of wound care and wound dressing products can reduce the risk of infection, as contamination of acute wounds can hinder the healing process. The selection of appropriate dressings to protect the wound from microorganisms and the length of dressing changes is adjusted to the healing process and the amount of exudate released [1].

Diabetic wounds are initially categorized as ordinary wounds, but if the management of the wound is not appropriate, it will experience infection, ulceration, and gangrene [2]. Diabetic ulcers cause erosion of the skin starting from the dermis layer to deeper tissues. If good care is not taken, the ulcer healing process will take a long time, and peripheral neuropathies can even occur which resulting in amputation. This condition can affect a person's quality of life [3] [4].

Wound infection goes through three stages: contamination, colonization and infection. Wound contamination is the presence of microbes that do not replicate in open wounds. Relatively small numbers of microbes do not affect the inflammatory response and wound healing process, but if microbial replication leads to colonization, there is a risk of infection. Increased microbial numbers can prolong the inflammatory phase and further tissue damage. When microbes migrate deep into the wound and multiply rapidly, it triggers infection [5]

Wound care is currently developing. The principle of wound care is the moist wound dressing technique, where the wound is kept moist. The purpose of keeping the wound moist is to support the wound healing process, maintain the risk of tissue fluid loss and cell death. Ulcers in moist conditions help wound healing up to 45%. The results of diabetic ulcer treatment for 7 days found no signs of infection, improved tissue perfusion, and wound healing process [6]

Aloe vera functions as an antiseptic and protects the skin from dehydration. The results of the application of aloe propolis on diabetic ulcers for 10 days of treatment of ulcer conditions improved and there were no signs of infection [7]. Garamycin is a class of bactericidal drugs that have antimicrobial properties. The results of the NaCl 0.9% + Gentamicin sulfate 80 mg and 160 mg irrigation study effectively prevented the occurrence of surgical site infections in orthopedic elective surgery patients compared to NaCl 0.9% alone [8]. This study aimed to determine the effectiveness of 0.9% NaCl compress and aloe vera gel with 0.9% NaCl and garamycin on acute wounds of diabetic gangrene rats.

METHODS

The study used experimental methods with acute wounds of diabetic gangrene rats [9]. This research was conducted in August 2024 at the Microbiology Laboratory of Bhakti Kencana University. The sampling formula uses the Federer formula. Federer's formula is a formula that is often used to calculate samples with experimental research [10]. The number of samples was 16 divided by 2 treatment groups, so that each group had 8 Wistar rats. The rats used are rats that are conditioned to experience diabetes mellitus by being given the drug Alloxan. Wistar rats were checked for blood sugar levels using

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GCU glucose sticks. Then give alloxan induction at a dose of 120 mg/KgBB intraperitoneally. After 72 hours or 3 days, the blood sugar levels of animals that have been induced by alloxan are checked again. Normal rat blood sugar levels are 50- 135 mg/dl and are said to be hyperglycaemic if the blood sugar levels of rats > 135 mg/dL [11]. After known sugar levels > 135 mg/dL, which will be made acute wounds and the treatment carried out is in the treatment group one with wound care moist dressing using NaCl 0.9% plus aloe vera, and treatment group 2 with wound care moist dressing using NaCl 0.9% plus garamycin. Wound care was carried out for 7 days.

Evaluation to determine the condition of the wound by examining the number of bacteria before and after treatment. The instrument used in data collection is an observation sheet. This observation sheet is used for several things, namely to record the blood glucose levels of rats after being given alloxan until the condition of the rats is maintained diabeticum, to record the condition of the wound, and to record the number of bacterial colonies shortly after an acute wound is made by smearing the diabetic rat's wound. This observation sheet is to record changes in acute wounds during treatment using moist dressings of a 0.9% NaCl mixture with Aloe vera and 0.9% NaCl with garamycin. In addition to wound care, the observation sheet also recorded that the blood glucose levels of rats remained above the value > 135 mg/dL by giving alloxan to keep the rats in diabetic condition. This study was assisted by enumerators to perform wound care every morning and evening. While counting the number of bacterial colonies was counted by laboratory experts. The data obtained will be tested for normality. For samples less than 50 normality test with the Shapiro-Wilk test. This study used 16 rats, so the normality test with Shapiro-Wilk. If the results are obtained as normally distributed data using a paired t-test, but if the data is not normally distributed using Wilcoxon test.[10][11].

This study used wistar rats with acute diabetic gangrene wounds. Before the research began, a submission was made to the Health Research Ethics Commission (KEPK) of Bhakti Kencana University to obtain a letter of ethical feasibility with Ethical Approval No. 138/09.KEPK/UBK/VII/2024. The study was conducted for 2 weeks, starting with 4 days of adapting the rats to the new environment in a clean cage and given food. Then the next 3 days were given alloxan to increase the blood glucose levels of rats, and the last 7 days of rat treatment were conducted according to predetermined research procedures. The experimental animals were fed and their cages were cleaned every day until the study was completed. After completion of the study, the experimental animals were killed by putting the rats in a box given carbon dioxide gas for 10 minutes.

RESULT

Table 1. Number of Bacterial Colonies Before and After Wound Treatment in Gangrene Rats with 0.9% NaCl compress and Aloe Vera

No	Rat Group	Number of Bacterial Colonies Before Treatment	Number of Bacterial Colonies After Treatment	Average reduction in the number of bacterial colonies	Percentage (%) reduction in the number of bacteria before and after treatment
1	K1 Rat 1	263	83	180	68,44
2	K1 Rat 2	255	69	186	72,94
3	K1 Rat 3	314	121	193	61,46
4	K1 Rat 4	294	228	66	22,45
5	K1 Rat 5	273	81	192	70,33
6	K1 Rat 6	221	94	127	57,47
7	K1 Rat 7	232	81	151	65,09
8	K1 Rat 8	190	110	80	42,11

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	Total number of colonies	2042	867	1175	57,54
	Average	255,25	108,375	146,875	57,54

Based on Table 1, all rats showed a decrease in the number of bacterial colonies after wound treatment with a combination of NaCl 0.9% and Aloe vera.
Data obtained from the results of the study were tested for normality. Because the number of samples is less than 30, the data normality test is performed using the Shapiro-Wilk test.

Table 2. Normality Test Results

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
Residual_Aloe vera	Statistic	df	Sig.	Statistic	df	Sig.
	.255	8	.135	.818	8	.044

a Lilliefors Significance Correction

Based on Table 2, the treatment of 0.9% NaCl and aloe vera obtained a significance value of 0.044 (<0.05). It can be concluded that the data is not normally distributed, so the analysis test to determine the effect of the treatment of 0.9% NaCl and aloe vera using the Wilcoxon test.

Table 3. Wilcoxon Test Results

Test Statistics	
	AfterAloe vera – BeforeAloe vera
Z	-2.521[b]
Asymp. Sig. (2-tailed)	.012
a. Wilcoxon Signed Ranks Test	
b. Based on positive ranks.	

Based on Table 3, the p-value (Asymp. Sig. 2-tailed) is 0.012, meaning that $p < 0.05$ H0 is rejected, so the results show that there is a statistically significant difference between the number of bacterial colonies before and after treatment, meaning that treatment with aloe vera and NaCl 0.9% is effective in reducing the number of bacterial colonies.

Table 4. Number of Bacterial Colonies Before and After Moist Dressing Wound Care with 0,9% NaCl + Garamycin in Rats with Acute Gangrene Diabeticum Wound

No	Rat Group	Number of Bacterial Colonies Before Treatment	Number of Bacterial Colonies After Treatment	Average reduction in the number of bacterial colonies	Percentage (%) reduction in the number of bacteria before and after treatment
1	K2 Rat 1	425	103	322	75,76
2	K2 Rat 2	287	77	210	73,17
3	K2 Rat 3	293	79	214	73,04
4	K2 Rat 4	289	89	200	69,20
5	K2 Rat 5	274	63	211	77,01
6	K2 Rat 6	455	43	412	90,55
7	K2 Rat 7	187	62	125	66,84
8	K2 Rat 8	123	42	81	65,85
	Total number of colonies	2333	558	1775	76,08
	Average	291,625	69,75	221,875	76,08

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Based on Table 4, all rats showed a decrease in the number of bacterial colonies after wound treatment with a combination of 0.9% NaCl + garamycin.

Table 5. Normality Test Results

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Residual_Garamycin	.146	8	.200*	.952	8	.733

*. This is a lower bound of the true significance.
a. Lilliefors Significance Correction

Based on Table 5, the treatment of 0.9% NaCl and garamycin obtained a significance value of 0, 733 (> from 0.05), so the data is normally distributed, then the analysis test to determine the effect of the treatment of 0.9% NaCl+garamycin using the Paired T Test.

Table 6 Paired T-Test Results

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Before Garamycin – After Garamycin	221.875	104.337	36.889	134.647	309.103	6.015	7	.001

Based on table 6, the Sig value. (2-tailed) 0.001 <0.05, then there is a significant difference in the number of bacterial colonies before and after being treated with wound care with 0.9% NaCl and garamicyn.

DISCUSSION

Based on the results of the study in Table 1, the number of bacterial colonies decreased after treatment for 7 days every morning and evening with 0.9% NaCl compress moist dressing technique and aloe vera. Diabetes mellitus can cause various acute and chronic complications. Diabetic ulcers are open wounds on the skin surface due to macroangiopathic complications resulting in vascular insufficiency and neuropathy, and can develop into infections due to the entry of bacteria and the presence of high blood sugar, which is a strategic place for germ growth. Bacteria found in diabetic ulcers are a combination of aerobic and anaerobic bacteria [12],[13].

This is in line with wound care research with moist techniques. This technique can facilitate cell growth and collagen proliferation in a healthy non-cellular matrix in the wound, so that tissue integrity can be improved [14]. The selection of Aloe vera gel as a non-pharmacological therapy for ulcers has advantages, among others, materials that are easy to apply to the skin, easily available in the market, and a price that is quite affordable. Aloe vera contains glucomannan as a driver of the growth of cells damaged by wounds and shrinks tissue. The content of oxidase, catalase, and lipase enzymes in aloe vera gel helps break down bacteria so that it can act as an antibiotic and reduce pain [15][16]. In contrast to the results of research comparing the effectiveness of wound care with Metronidazole and NaCl 0.9%, it was found that the results of diabetic ulcer wound care using NaCl 0.9% solution showed that NaCl 0.9% compresses had effectiveness on diabetic ulcer wound healing, even though the decrease was only 8.19 compared to using metronidazole[17].

Wound care methods with dressings in the form of gauze and 0.9% NaCl solution are considered less effective because the nature of 0.9% NaCl will evaporate so that the gauze becomes dry and sticks to the wound [18],[19]. Wound treatment with a combination of 0.9% NaCl compresses and aloe vera can maintain the moisture of the gauze and the content of aloe vera as an antiseptic. This affects the condition of acute

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wounds in diabetic gangrene rats, the number of bacterial colonies decreases during 7-day treatment. The wound condition on the 7th day became dry. Table 3 Wilcoxon test results 0.012 <0.05. There was a statistically significant difference between the number of bacterial colonies before and after treatment with NaCl 0.9% + aloe vera. The novelty of this study is wound care in diabetic gangrene rats with a combination of 0.9% NaCl compresses and aloe vera, so that moisture can be maintained, and there is an antiseptic in aloe vera content. Acute wound parameters improved after 7 days of treatment, indicated by a decrease in the number of bacterial colonies.

Based on Table 4, all rats showed a decrease in the number of bacterial colonies after wound treatment with a combination of NaCl 0.9% + Garamycin. Wound treatment carried out for 7 days every morning and evening with NaCL 0.9% and Garamycin showed a decrease in the number of bacterial colonies. These results are in line with previous research conducted on acute orthopaedic wounds found no increase in the number of microbial colonies compared to 0.9% NaCl compress treatment alone[20]. Garamycin is a class of bactericidal drugs that have antimicrobial properties, so that acute wound treatment with 0.9% NaCl compress added with 80 mg garamicyn can prevent bacterial growth. This can be seen from the average decrease in the number of bacterial colonies after wound treatment with the moist technique in rats with acute diabetic gangrene wounds. Table 6 paired T-test results) p value 0.001 <0.05, then there is a significant difference in the number of bacterial colonies before and after being treated with wound care by administering NaCl 0.9% and garamicyn. Acute diabetic gangrene wounds if not treated properly, can progress to chronic wounds. Bacterial colonies will increase in number, influenced by environmental hygiene and the presence of a breeding ground for bacteria. The media for breeding bacteria are those that have nutrients derived from, among others, carbohydrates [12]. Impaired healing in diabetes is the result of a complex pathophysiology involving vascular, neuropathic, immune, and biochemical components. Hyperglycemia correlates with stiffer blood vessels, which cause slower circulation and microvascular dysfunction, causing reduced tissue oxygenation. Blood vessel alterations observed in diabetic patients also account for reduced leukocyte migration into the wound, which becomes more vulnerable to infections [21] The addition of garamicyn as an anti-bacterial can help suppress the growth of the number of bacteria in acute wound care of rats with diabetic gangrene[22]

Based on table 3 and table 6, the significance of the paired sample test (0.001) while the significance of the Wilcoxon test (0.012), the test results show that the significance value of the paired sample test is smaller than the Wilcoxon test, so that wound treatment with NaCl 0.9% + garamicyn is more effective than NaCl 0.9% + aloe vera. The novelty of this research lies in comparing moist dressing principles for diabetic ulcers by quantifying bacterial colonies. This study has limitations, especially the frequent removal of the dressing, because the hair on the shaved rats makes it difficult for researchers to maintain the plaster glued to the rat's skin. The solution was to fix the dressing using rolled gauze on the rat's body.

CONCLUSION

There is a statistically significant difference between the number of bacterial colonies before and after wound treatment of diabetic gangrene rats with NaCl 0.9% plus aloe vera and 0.9% NaCl and garamicyn. Wound treatment of diabetic gangrene rats with NaCl 0.9% + garamicyn is more effective (76,08%) than NaCl 0.9% plus aloe vera (57,54%). Recommendation further research is needed in humans with acute diabetic ulcers.

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