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Effect of red fruit oil soap (*Pandanus conoideus* LAM) as wound cleansing on wound healing and the number of bacterial colonies among Grade II Diabetic Ulcer Patients at Griya Qound Care Clinic Kudus, Indonesia

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ABSTRACT

Background: Diabetic ulcer is an open wound on the skin layer to the dermis due to hyperglycemia and neuropathy. This condition often causes infection and becomes an inhibitor in the wound healing process. Red fruit oil soap can be used as an alternative cleansing to reduce the number of bacterial colonies and accelerate the wound healing process. This present study aimed to observe the effect of red fruit oil soap in the cleansing process to reduce the number of bacterial colonies and the wound healing process in grade II Diabetic ulcer patients

Methods: This is a quasi-experimental study with the pre-test post-test non-equivalent control group design. Wound cleansing in the intervention group used 0.9% NaCl solution and red fruit oil soap with a pH of 5.74, while the wound cleansing in the control group used 0.9% NaCl solution. Bacterial colonies were assessed and the type of bacteria was observed by the swab method. The wound healing was assessed with Bates-Jensen Wound Assessment Tool (BWAT) instrument for 14 days with observations every 3 days. Analysis test used Mann Whitney and Repeated Measure ANOVA.

Results: There was no difference in the mean of the number of bacterial colonies between the wounds that were cleansed with 0.9% NaCl solution and red fruit oil soap and the wounds which were cleansed by using 0.9% NaCl solution ($p > 0.05$). The mean of the number of colonies in the intervention group until the 14th day reduced by 3.14x106 and in the control group was reduced h 1.40x106. There was a significant decrease in the wound healing scores in each group, in each assessment for 14 days.

Conclusion: This study found that the wound cleansing using red fruit oil soap and 0.9% NaCl solution could reduce the number of bacterial colonies on the wound surface and accelerate the wound healing process among patients with grade II diabetic ulcer.

Keywords: Red fruit oil soap, Wound cleansing, Wound healing, Diabetic ulcer
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1. Introduction

Diabetes Mellitus (DM) is a chronic metabolic disease characterized by hyperglycaemia due to a decrease in the level of the insulin hormone. Indonesia is the 7th largest with the number of DM patients in the world where an estimated of 21.3 million adults suffered from DM in 2013. The increasing incidence of DM usually will be followed by the increased incidence of diabetic ulcers. It is estimated, 15% -25% of people with diabetes will experience diabetic ulcers during their lives and the condition of the ulcer will be worse if the ulcer is infected [1].

There are five basic principles in DM management, namely engaging in low-carb DM diet, routine physical activity (exercise), taking Oral Hyperglycaemia Drug (OHD) or insulin, routine health check and blood glucose level monitoring to healthcare services, and stress management [2]. The proper management of DM is necessary in order to prevent complications such as macroangiopathy and microangiopathy. The most frequent complication is the occurrence of pathological changes in the lower limbs called diabetic ulcers which are often not felt and can develop into infections caused by aerobic

or anaerobic bacteria. Diabetic ulcers is one of the most severe and disabling complications of DM because the tissue damages that occurs in diabetic foot ulcers is caused by neurological (neuropathy) and vascular disorders on the legs.

DM patients who are accompanied by diabetic ulcer complications not only require medical management but also a holistic management including metabolic, vascular, infection, ulcer, pressure/ mechanical, and institutional control. Ulcer control as the most critical intervention in ulcer treatment is a wound treatment by removing infected tissue and necrosis regularly. The wound treatment itself comprised of three stages namely cleansing, debridement, and dressing [3]. Cleansing is the initial stage in wound care which plays a vital role in maintaining the cleanliness of germs in the wound area, removing debris, exudate, dead tissue from the wound surface, minimizing bacterial colonization and facilitating wound healing. Showering technique is the most common methods in cleansing, which normally use 0.9% NaCl. Unfortunately, 0.9% NaCl does not have specific anti-bacterial power and thus, makes it less appropriate to be applied in people with infected diabetic foot ulcer such as diabetic ulcer, especially in grade II DM [4].

Red fruit contains beta crypto samine, phenolic compounds, triterpenoids (essential oils), steroids, phenylpropanoid glycosides, coumarin, chlorogenic acid, caffeic acid, squalene, phytosterol, and stigmasterol which functioned as anti-bacterial and anti-inflammatory which will damage the bacterial membrane and kill the bacteria [5, 6]. The active ingredient of the red fruit can be used as a cleansing solution for DM ulcers because it aims to reduce the number of bacterial colonies and accelerate the process of wound healing to optimize this function.

Soap is a salt compound of high fatty acids, such as sodium stearate, C17H35COONa+. Cleansing action of soap is produced by emulsifying forces and the ability to reduce surface tension from the water. This concept can be understood by remembering the two properties of soap anions [7]. Antiseptic soap is soap with the addition of chemical compounds used to kill or inhibit the growth of microorganisms in living tissue such as the surface of the skin and mucous membranes. Ordinary soap requires rubbing to mechanically remove microorganisms, while antiseptic soap (antimicrobial) can remove and also more effective to kill or inhibit the growth of most microorganisms (broad range) than detergent or plain soap [8].

Considering the potential of red fruit as the wound cleaning and wound healing materials, it is interesting to develop a study with the presence of new creativity and innovation related to red fruit oil soap to reduce the number of bacterial colonies, as a wound cleansing in the process of ulcer healing among the grade II diabetic ulcer patients at Griya Wound Care Clinic Kudus. The present study would be the first soap made from red fruit oil which is used as a cleansing before performing wound care by using modern topical dressing.

2. Method

This study used the quasi-experimental method with the pre-test post-test non-equivalent control group design. This type of study design was used to analyse red fruit oil soap as a wound cleansing agent for wound healing and decrease the number of bacterial colonies in grade II diabetic ulcer patients. The study samples were all DM patients with a complication of grade II diabetic ulcer with a wound size of at least 4 cm2 and were treated during March-April 2018 at Griya Wound Care Clinic Kudus, Indonesia. There were 27 respondents involved in the study, of whom, 15 respondents were assigned as the intervention group and 12 respondents as the control group. The intervention group was administered at wound surface with red fruit oil soap and 0.9% NaCl solution as cleansing treatment whereas the control group received ulcer cleansing treatment using 0.9% NaCl solution only.

The red fruit oil soap was prepared by the researchers with the guidance of a herb specialist. The soap was made from red fruit oil with 250gr of 0.9% NaCl powder and 750gr of texapone dissolved in distilled water. Viscosity, pH, foam power, and irritation test were performed in a university's laboratory while the soap's power in inhabiting and killing bacteria was performed in the Central Java Laboratory. Data collection was carried out by pre-test and post-test using BWAT (Bates-Jensen Wound Assessment Tool) instrument and tissue culture was collected to observe the number of bacterial colonies. The bacterial pus colony culture was collected using the swabbing technique using

sterile cotton swabs with dry evaporation above 100^{o}C for 30 minutes. The ulcer was moistened with NaCl 0.9%, swabbing was performed zigzag until covered all ulcer areas. Cotton swabs were immediately put into sterile bottles that contained 3 cc of 0.9% NaCl solution in closed test tubes to prevent it from drying out, and on the outer tube was labeled with patient identity. The thee were immediately sent to the laboratory using the cool box for the examination process.

Repeated cleansing and ulcer treatments were conducted every 3 days while colonies observation and ulcer healing were assessed using BWAT tools for five times (Day 1, 3,7,10 and 14) in a duration of 14 days of observation. Higher score of BWAT indicating a poor ulcer healing process (wound degeneration) while the lower score indicating wound regeneration.

Considering that blood glucose affects the wound healing process, measurements of this confounding variable was also performed following the treatment. Examination of the number of bacterial colonies was conducted by pus culture with swabbing technique to observe the number of bacterial colonies while the ulcer healing process observation was conducted before and after the wound cleansing intervention. Examination of study samples to determine the number of bacterial colonies was carried out at the Central Java Laboratory, Indonesia. Analysis of the results of the wound healing process was performed using Repeated General Linear Model and Pos Hoc tests, whereas the number of bacterial colonies was assessed by Friedman and Post Hoc and Man Whitney. This study has received an Ethical Clearance from the Education Institution of Semarang Health Polytechnic, Indonesia.

3. Results

3.1 Demographic characteristics

The characteristics of the respondents between intervention and control group in this study was relatively homogeneous (see Table 1). Most of respondents in both groups were older person aged 55-64 years old with the mean age of 55. The proportion of male-female was different, where the males dominate control group, however, the females dominate intervention group. The vast majority of respondents both in the control and intervention groups did not have regular exercise (93% in the intervention and 100% in the control group) and engaged in regular smoking (66% in the intervention and 51.9% in the control group). For co-morbidities, the majority of respondents had non-DM comorbidities, such as renal failure, visual impairment, hypertension, and peptic ulcer.

3.2 Blood Glucose

The blood glucose of DM patients with a complication of grade II diabetic ulcer in the intervention and control group were mostly above the normal level (>200 mg/dl). Although the homogeneity test results on the five blood glucose measurements also showed that there was no difference of the data variants between the control group and the intervention group, however, Table 2 showed that the blood glucose levels of respondents in the control group were generally lower than their counterparts in the intervention group. In both intervention and control group, there is a tendency of decreasing blood glucose level where the blood glucose levels in the latest day of observation were at the lowest compared to the initial period.

3.3 Bacterial Colonies

The number of bacterial colonies of patients of the control group in the initial period (Day 1) was lower compared to their counterparts in the intervention group (see Table 3). The number of bacterial colonies in the control group and the intervention group for 14 days of study with 5 times assessment showed a gradual decrease in the number of bacterial colonies but still in a state of infection (>10^5). However, Table 3 also showed that the number of bacterial colonies in the intervention group significantly increased in the last day of observation.

Independent t-test Mann-Whitney (see Table 4) showed that that there was no difference in bacterial colonies on the ulcer surface between groups at each assessment. However clinically, the number

Table 1. Frequency distribution of respondents' characteristics based on demographic characteristics in the control group and intervention group

Characteristic	Control Group (n=12)		Intervention Group (n=15)		Total		*p
	n	%	n	%	n	%	
Age							0.724*
45-54	5	41.7	7	46.7	12	44.4	
55-64	6	50.0	7	46.7	13	46.1	
64-75	1	8.3	1	6.7	2	7.4	
Gender							0.495*
Male	8	66.7	6	40.0	14	51.9	
Female	4	33.3	9	60.0	13	48.1	
Regular Exercise							1.000*
Yes	0	0	1	6.7	1	3.7	
No	12	100	14	93.3	26	96.3	
Co-morbidities							0.948*
Without co-morbidities (DM)	4	33.3	4	26.7	8	29.6	
Renal failure	-	-	1	6.7	1	3.7	
Glaucoma	2	16.7	1	6.7	3	11.1	
Hypertension	2	16.7	4	26.7	6	22.2	
Cataract	4	33.3	4	26.7	8	29.9	
Peptic ulcer	-	-	1	6.7	1	3.7	
Antibiotic							0.59*
Ciprofloxacin 500 mg	8	66.7	8	53.3	16	59.2	
Metronidazole 500 mg	4	33.3	7	46.7	11	40.8	

*Homogenity Test: *Leven's Test*

of colonies in both the control group and the intervention group experienced a decrease in each assessment with the final number of below 10^7.

3.4 Ulcer healing

Figure 1 showed a description of Tthe ulcer healing process both in the control group and the intervention group withshowed an ulcer improvement (wound degeneration). It can be seen from, the decrease of ulcer score in each because the ulcer score always decreased in each assessment, but unfortunatelyit had not reached the remodeling stage.

Repeated measure Post Hoc Bonferroni Analysis showed a p-value of <0.05 in each day of measurement, indicating a difference in wound healing scores between the control and the intervention group. BWAT observation scores illustrated the wound healing process for grade II diabetic ulcer patients who received red fruit oil soap and the group treated by using 0.9% NaCl solution (intervention group) progressed better (wound regeneration) compared to patients in the control group whose wound only cleansed using 0.9% NaCl solution (see Table 5).

4. Discussion

A diabetic ulcer is one of the chronic ulcers and is known as difficult to heal. Since the improvement of the healing process is slower and longer, the evaluation of ulcer improvement can be done every 3 days along with ulcer treatment. In this study, evaluation or assessment of ulcer healing status was conducted 5 times in 14 days, i.e. every intervention with cleansing and ulcer treatment as well as assessments before (pre) and after (post) intervention. The first pre and post-tests were conducted on

Table 2. Frequency distribution of blood glucose in the control group and intervention group

Blood Glucose	Control Group (n=12)		Intervention Group (n=15)		*p
	Mean±SD	Min-Max	Mean±SD	Min-Max	
Day 1	270.50±43.617	189-345	337.07±89.515	95-445	0.097
Day 3	243.25±45.383	165-303	305.20±96.295	90-492	0.090
Day 7	211.25±39.651	147-275	279.40±78.656	84-380	0.115
Day 10	187.33±37.655	133-253	247.47±71.109	121-412	0.112
Day 14	156.58±38.030	101-201	210.40±70.440	103-380	0.139

*Homogenity Test: *Levene Test*

Table 3. Number of Bacterial Colonies before and after wound cleansing in the control and intervention group

Day of Assessment	Control Group (n=12)		Intervention Group (n=15)	
	Mean		Mean	
	Pre	Post	Pre	Post
Day 1	$3.61x10^7$	$3.18x10^7$	$8.21x10^6$	$7.08x10^6$
Day 3	$2.56x10^7$	$2.48x10^7$	$1.18x10^7$	$5.74x10^6$
Day 7	$3.03x10^7$	$2.47x10^7$	$6.69x10^6$	$4.11x10^6$
Day 10	$2.72x10^7$	$2.45x10^7$	$6.33x10^6$	$2.13x10^6$
Day 14	$2.77x10^7$	$2.63x10^7$	$4.03x10^6$	$9.24x10^5$

day 1, second pre and post-tests were conducted on day 3, third pre and the post-tests were conducted on day 7, fourth pre and post-tests were conducted on day 10, fifth pre and post-tests were conducted on day 14.

4.1 Bacterial colonies

In addition to evaluating the ulcer healing process using the Bates-Jensen Wound Assessment Tool (BWAT), the authors also evaluated the number of bacterial colonies on the ulcer surface. Gram positive and negative bacteria were found at the pus samples in DM ulcer patients examined for bacterial colonies. Gram-positive bacteria include Staphylococcus epidermis, while negative gram bacteria include Enterobacter aerogenes, Proteus mirabilis, and Klebsiella spp [9]. Infection with respect to superficial ulcers is most often caused by aerobic gram-positive bacteria while aerobic and anaerobe gram bacteria are rare to find in deep ulcers which are usually considered due to mixed infections. Anaerobic bacterial infections are generally associated with tissue necrosis and osteomyelitis [10].

The study found that the number of bacteria on the ulcer surface decreased, and it was applied both in the control group and the intervention group. There was no significant difference in the mean number of bacteria before and after treatment in each assessment between the groups. The final assessment results of bacterial colonies still showed the number of above 105, indicating the wound was still in the inflammatory phase. However, it can be seen from the mean value and the difference in the assessment results of bacterial colonies carried out 5 times during 14 days showed a decrease. In the intervention group where the ulcer was cleansed using 0.9% NaCl solution and red fruit soap showed a higher decrease than the ulcer that was cleansed only by using 0.9% NaCl solution.

One of the substances contained in red fruit is essential oils that have antibacterial properties [11]. Essential oils in red fruit oil are in the form of triterpenoid compounds which carbon frame is derived from six isoprene units and biosynthetically derived from acyclic C-30 hydrocarbons, namely squalene. Triterpenoid compounds consist of four groups, namely steroids, saponins, and triterpenes. Steroids are triterpenoid compounds that act as an anti-inflammatory [5]. Squalene is a high-saturated aliphatic hydrocarbon that includes triterpenes. This compound has the potential to enter the body through the skin by rubbing it on the surface of the skin, and this compound can be absorbed within 0.5 seconds, spread over 1 mm and in less than 1 minute to reach the blood vessels, so this compound is very good for skin health [12]. Besides, red fruit also contains phenylpropanoid glycosides which work by damaging the bacterial membrane that can kill the bacteria [13].

Table 4. Differences in the number of bacterial colonies on the ulcer surface in control group and intervention group

The difference in the number of bacterial colonies	Group	Mean difference	*p
Day 1	Intervention	$1.13x10^6$	0.283
	Control	$4.30x10^6$	
Day 3	Intervention	$6.05x10^6$	0.608
	Control	$7.98x10^5$	
Day 7	Intervention	$2.58x10^6$	0.558
	Control	$5.59x10^6$	
Day 10	Intervention	$4.20x10^6$	0.272
	Control	$2.65x10^6$	
Day 14	Intervention	$3.10x10^6$	0.283
	Control	$1.40x10^6$	

*Independent Test:
Man-Whitney

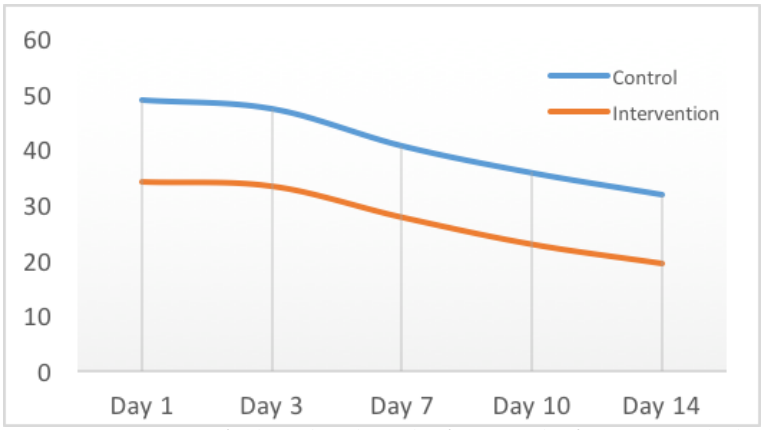

Figure 1. Description of ulcer healing before and after wound cleansing

There was no difference in the number of bacterial colonies and it was likely due to age, systemic disease, immune disorders, and consumption of immunosuppressant. In this study, the mean of age was 55-year-old which showed that the respondents were in the elderly adult phase with deteriorated body function, coupled with the DM disease so that the un-optimal immune function resulted in increased bacterial growth.

4.2 Healing process of diabetic ulcers

Statistical test result using Repeated Measure General Linear Model (GLM) ANOVA showed that there was a significant difference in BWAT scores between the control group and the intervention group which illustrated the healing process of a diabetic ulcer on each assessment ($p < 0.05$). In both groups, ulcer healing scores decreased during the 14 days of the study, but the ulcer had not reached the maturation stage. The group that was cleansed using red fruit oil soap showed a decrease in the number of bacterial colonies more than the group cleansed only with 0.9% NaCl solution.

In addition to the antibacterial property possessed by red fruit oil soap, red fruit also contains vitamins C, E (tocopherol) and A which have a role in collagen synthesis, fibroblast proliferation, and angiogenesis so as to accelerate the process of ulcer healing [14, 13]. Vitamin E also plays a role in the healing process of the ulcer by protecting the cell membrane from damage caused by oxidant substances. Red fruit also contains minerals such as Zinc (Zn) and iron (Fe) which play a role in ulcer healing. Zn plays a role by increasing collagen and protein synthesis, and increasing tissue growth. Whereas iron plays a role in providing oxygen for tissue that has injury or ulcer, and also plays a role in collagen synthesis and strengthens new tissue that is formed after the ulcer healing.

In this study modern dressing was used based on the wound bed and the state of the patient's

Table 5. Differences in BWAT scores between the control group and intervention group

BWAT Score	Variable Group	Mean±SD	*p
Day 1	Control	48.92±2.275	0.001
	Intervention	34.20±4.902	
Day 3	Control	47.33±2.348	0.001
	Intervention	33.40±4.672	
Day 7	Control	40.67±3.339	0.001
	Intervention	27.73±4.183	
Day 10	Control	35.83±2.823	0.001
	Intervention	22.80±2.597	
Day 14	Control	31.92±2.712	0.001
	Intervention	19.13±2.748	

ulcer. The type of modern dressing used was hydrogel in the form of metcovazin and dermozone salt which usually used for wound care management in Indonesia [15]. Metcovazin salt is a salt with the active ingredient of iodine-cadexomer which has antibacterial property, besides it has a function to support autolysis debridement, maintain a moist atmosphere, and stimulate the growth of granulation tissue. Meanwhile demozone salt is a salt with an active ingredient and dalethyne compound derived from ozone-treated olive oil which is able to kill MRSA bacteria and help bactericidal processes and has anti-inflammatory property.

From the score of the ulcer assessment at baseline (the first time the respondents arrived), all respondents were in the phase of degenerative ulcer (the score was more than 13) where the ulcer was in the inflammatory phase. The duration of the ulcer healing process is related to the body's ability to respond to infection by metabolizing to produce materials that fight the bacteria, especially white blood cells and nutrients for the continuation of ulcer healing. The state of blood glucose in the respondent also affected the body's metabolism so that the components that should be produced by the body and transported through the blood vessels became disrupted due to metabolic disorders due to hyperglycemia [16]. According to a study the presence of necrotic tissue, devitalization tissue and high infection of the ulcer resulted in an excess increase in metabolism which caused tissue hypoxia [17].

The occurrence of tissue hypoxia results in the damaged extracellular matrix and fibroblast formation for ulcer healing will be disrupted which causes the longer duration of the ulcer healing. The longer duration of ulcer healing is due to macrophage cells identify hypoxic cells as cells that no longer function for phagocytosis. This causes the time for macrophages to do phagocytosis of bacteria or unhealthy tissue in the framework of autolytic debridement and for the subsequent process to increase in length, so that the fibroblasts cannot perform their functions maximally [17].

The choice of dressing is a technique in creating a moist ulcer environment. This is found in ulcer care management (TIME). The results of a systematic review conducted by Dumville et al (2012) on modern dressing for diabetic ulcer healing, stated that the selection of bandages in accordance with evidence-based practice could accelerate ulcer healing [18]. The results of this study differed from Julianto's (2014) study, which found that the improvement of ulcers treated using NaCl solution was 0.9% lower than those treated with hydrogel [19]. A study conducted by Paradika (2016) also found that ulcers cleansed with 0.9% NaCl solution had a longer healing duration compared to ozone water-treated ulcers [20].

Changes in wound conditions that can be observed directly include the reduced amounts of exudate from many to a little, necrotic tissue with only a small amount of exudate, skin color around the wound is bright red and the growth of granulation tissue and epithelialization which show good progress. Granulation tissue at the time of the pre-test was not visible or only <23%, with the pale red of 78, while the tissue growth in post-test III was <75% with light and bright red. Meanwhile the wound closure by epithelial tissue at pre-test was <25% and became 25-49% in post-test III. Acceleration of granulation tissue growth and epithelialization is determined with good wound control and also influenced by a high protein diet such as consuming more than 4 eggs per day and consuming snakehead fish rich in protein content.

The condition of the ulcer that was cleaned using 0.9% NaCl solution and red fruit oil soap showed that the ulcer was in the granulation phase, with the clinical condition of 75% granulation tissue growth, the ulcer edge began to show contractions and clear border, no erythema, induration, and the skin color around the ulcer was normal with minimal exudate production and the ulcer surface was covered with necrotic tissue <25%. Based on this description, ulcer treatment with cleansing using 0.9% NaCl solution and red fruit oil soap by maintaining the moist condition of the ulcer with the right choice of dressing according to wound bed was very likely to be applied to accelerate the healing process of grade II diabetic ulcer in the inflammatory phase even though the observation on Day 14 showed that it had not reached the maturation phase. Thus, the clinical application of ulcer treatment with cleansing using 0.9% NaCl solution and red fruit oil soap should be conducted until the ulcer reached the maturation phase accompanied by the selection of the appropriate dressing.

5. Conclusion

Although there was no difference in the mean of the number of bacterial colonies between the wounds that were cleansed with 0.9% NaCl solution and red fruit oil soap, and the wounds which were only cleansed by using 0.9% NaCl solution ($p > 0.05$), the study found the mean of the number of colonies in the intervention group until the 14th day reduced by 3.14×10^6 and in the control group was only reduced by 1.40×10^6. The results indicating that wound cleansing using red fruit oil soap and 0.9% NaCl solution could reduce the number of bacterial colonies on the wound surface and accelerate the wound healing process among patients with grade II diabetic ulcer.

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