

THE EFFECTIVENESS OF RED BETEL LEAF (*Piper crocatum*) CLEANSING INFUSA IN REDUCING THE NUMBER OF TOTAL BACTERIA OF ULCER DIABETICUM ISOLATES IN ALLOXAN INDUCED WHITE RATS

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Abstract

Background: Diabetes mellitus (DM) is a chronic disease that can cause complication diabetic ulcers, which have a long healing process. The wound management is very important here, especially the cleansing of the wound. The choice of techniques and solutions is very important, swabbing and irrigation are frequently used techniques. Normal saline is a standard solution used for the cleansing process, but an alternative solution is still needed for cleansing. One of it is red betel leaf (*Piper crocatum*) infusa. Red betel leaf contains flavonoids, phenols, tannins, and essential oils that can used as antiseptic.

Objective: To determine the effectiveness of the using of red betel leaf infusion (*Piper crocatum*) in reducing the total bacterial number of diabetic ulcer isolates in white rats induced by alloxan.

Method: the design in this study was true pre post control experiment. The subjects in this study were winstar type white rats that induced by alloxan and carried out injuries. The number of samples of 20 rats were divided into 5 groups. Samples were treated using irrigation and swabbing techniques using 20% red betel leaves and normal saline infusion for up to 5 interventions, each bacterial number was calculated and then processed by one way ANOVA test analysis.

Results: the lowest bacterial number at the end of the intervention was 11 CFU / cm² and the highest was 143CFU / cm², the data were then analyzed using the ANOVA one-way test showing that the significance of cleansing with irrigation and swabbing techniques using 20% red betel leaf infusion. Results of Pos Hoc test with LSD obtained a p value of 0.001 for the cleaning group with a swabbing technique using 20% red betel leaf infusion.

Conclusion: in this study cleansing with irrigation technique using 20% red betel leaf infusion and swabbing using 20% red betel leaf infusion was effective in decreasing the total number of bacterial numbers of diabetic ulcer isolates in white rats induced by alloxan.

Keywords: red betel leaf, bacterial numbers, cleansing

INTRODUCTION

Diabetes mellitus (DM) or diabetes, is a chronic organ disorder, this condition is caused by an abnormality of the body to

produce insulin or an ineffectiveness of the body using insulin. According to the International Diabetes Federation (2012) there was 371 million people estimated have

diabetes and in 2030 it will able to reach 552 million, this can be illustrated there was 3 patient case per second (Cheng, 2013). In Indonesia it is estimated that in 2030 the prevalence of DM will reach 21.3 million people (Diabetes Care, 2004). Diabetes mellitus is the number 2 cause of death in urban areas and number 6 in rural areas, and the common DM that occurs is type 2 of DM (Ministry of Health, 2009).

Increasing the prevalence of DM will also increase the prevalence of diabetic ulcers, and these ulcers have risk of it ending with the infection and ending with amputation or death. Infection that occurs in DM ulcers can be caused by gram-positive or gram-negative bacteria. Microbes that found in DM ulcers in several studies are *Klebsiella* sp, *Proteus mirabilis*, *Staphylococcus aureus*, *Klebsiella*, *E. Coli*, *Pseudomonas aeruginosa* and *Alcaligenes faecalis*. (Decroli, et al., 2008)

The treatment of diabetic ulcer takes quite a long time, this causes quite expensive financing, moreover if there are complications occur or there are other diseases that accompany. Besides, there are some types of microbes that cause infections in diabetic ulcers that resistant/ have low sensitivity with several antibiotics. Research carried out by Decroli (2008) tells that the most sensitive antibiotics are carbapenem and netilmicin sulfate, but anaerobic microbes were resistant to this type of drug. The solution that is frequently used today for wound care is NaCl 0.9% or antibiotic solution. Therefore it is necessary to develop drugs that are used in wound care which are relatively cheaper but safe, as an alternative that can be developed is from plants. "Back to nature" is a term of the current pattern of life nowadays, but the use of traditional medicines must have strong foundations so that they are not threaten the community or

patients. The use of various types for wound care has been carried out to be an alternative choice. Red betel containing tannins, flavonoids, polenolol and saponen function as antibacterial which can be an alternative in dealing with infections that occur in diabetic wounds.

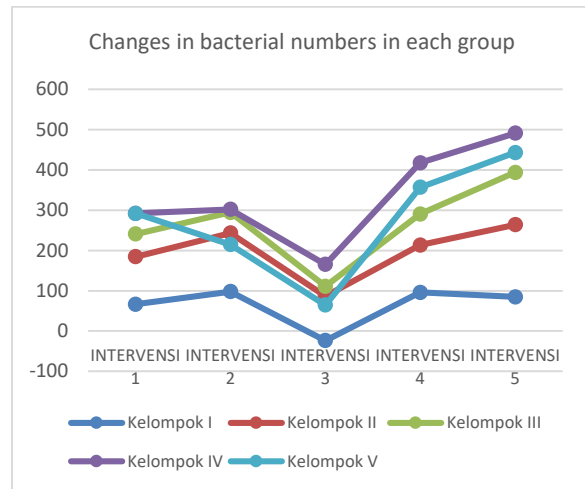
Wrong wound healing may be caused by cleansing process. Besides the type of solution that used, the technique that used for wound healing also can cause the wrong wound healing. In some literature, the cleansing method with irrigation is more recommended than the cleansing method with swabbing, this is because the swabbing method causes more damage to tissue granulation. Research on the use of red betel leaf infusion (*Piper crocatum*) for the treatment of DM ulcers has not been carried out, especially red betel leaf (*Piper crocatum*) which is used for cleansing, so this research is important in an effort to improve the treatment for diabetic ulcer.

RESEARCH METHODS

This research is a quantitative research that uses the true experiment method with a pre-post test control group design approach. The subjects in this study were winstar white rats. The number of samples 20 rats were divided into 5 groups, group I: cleansing with irrigation techniques using 20% red betel leaf infusion, II: cleansing with swabbing techniques using 20% red betel leaf infusion, III: cleaning with irrigation techniques using NaCl 0.9 %, IV: cleaning by swabbing the technique using 0.9% NaCl, V: Without treatment. Test animals are given induction of alloxan then it given a wound on his back. Wounds are treated every 3 days for up to 5 interventions, increasing the total bacterial counts before and continuing the intervention manually.

RESULTS

The number of bacteria was counted each before the cleansing and after the cleansing (pre-post) descriptively to find out the bacterial changes before cleansing and after cleansing, the data were processed by searching for delta means each intervention group with the results as shown in graph 1 below.



On the 28th day the number of bacteria in each group has the following results: group I at 57 CFU / cm², II at 11 CFU / cm², III at 84 CFU / cm², IV at 53 CFU / cm² and V at 131 CFU / cm². The highest number of bacteria in group V is equal to 143 and the lowest in group II is 11.

The results of data analysis with ANOVA test obtained p value <0.05 showed that cleansing with red betel leaf infusion was effective in reducing the total bacterial value of diabetic ulcer isolates in alloxan-induced white rats.

Post hoc test results with LSD cleansing technique and concentration of red betel leaf (*Piper crocatum*) infusion in the decreasing of total number of bacterial of diabetic ulcer isolates in induced alloxan white rats. Cleansing with the irrigation technique using 20% red betel leaf (*Piper crocatum*) infusion was obtained p <0, 05,

this showed that cleansing with irrigation techniques using a 20% red betel leaf (*Piper crocatum*) infusion is effective in decreasing the total bacterial number of diabetic ulcer isolates in alloxan-induced white rats (hypothesis 1 accepted). Cleansing with swabbing technique using 20% red betel leaf (*Piper crocatum*) infusion was obtained p <0.05, this indicated that cleansing with swabbing technique using 20% red betel leaf (*Piper crocatum*) infusion was effective to decreasing the number of total bacteria in diabetic ulcer isolates in alloxan-induced white rats (hypothesis 2 accepted).

DISCUSSION

Antimicrobial agents have several terminologies such as bacteriostatic, bactericidal, antibiotic and antiseptic. The difference from that term is how it works, like inhibits the growth of bacteria or kills bacteria. The action mode that can be carried out by microbial agents are DNA damage, protein denaturation, membrane or cell wall disruption, removal of loose sulphidyl groups and chemical antagonism. Cell membrane acts as a selective barrier, letting some solutions pass and rejecting others (Brooks, et al, 2001)

The process of fluid transfer is by diffusion, osmosis and active transport, which is a process that passes certain materials through membranes as a result of different compositions. Plasma membranes are semipermeable so only some materials can pass through this membrane. The transporting proses of liquids and solutes inside is also influenced by the concentration. If the concentration is low, then it will be easier to pass through semipermeable cell membranes, the higher the concentration, the transfer of fluids and solutes using other mechanism that require energy or active transportation (Juwono &

Juniarto, 2003). Also, the concentration of the red betel leaf infusion with a concentration of 20% of the content contained in the red betel leaf solution is able to decreasing the number of bacteria by the mode of action of the antimicrobial it has.

Research conducted by Juliantina, et al, (2009), is about the benefits of red betel as an anti-bacterial agent against gram-positive and gram-negative bacteria shows the effect of ethanol extraction of red betel has an antibacterial effect on gram-positive gram-negative bacteria. Essential oils, at low levels will form phenol proteins with weak bonds and immediately will be decompositioned, then phenols are added to cells and are further increased by protein denaturation. Proteins that have denaturation and coagulation will lose it physiological activity, so they cannot works properly. Changes in the structure of proteins in cell walls will increase cell permeability so that cell growth will be inhibited and then cells become damaged. At high levels of phenol it causes coagulation of proteins and membrane cells dependent on lysis. (Agustin, 2005; Juliantina, et al. 2009).

According to Kun et al. (2011), Klebsiella and Staphylococcus bacteria that contacted with tannin extract with a minimum dose after 24 hours will cause morphological changes in the form of shrinkage, dry, irregular, distorted cells and cell wall lysis. Tannin has the ability to precipitate protein, dissolves in the air. A similar study was carried out by Lim SH (2006) about the antimicrobial activity of hydrolyzed tannins on *R.apiculata*, after the exposure with tannins then appear to cause differences resulting from cell invagination cells that are stacked and then the cell wall lysis, this condition cause the leak of cytoplasm liquid. This changing can be connected with the character of the

hydrolyzed antimicrobial of tannins with gallic acid which affect the biosynthetic and synthesis steps of cell walls and cell membranes. Changes in cell membrane permeability can cause a decrease in cell volume as evidenced by the replacement of cell membranes from the cell wall. Betel leaf extract and red betel leaf content of 18%, effectively inhibits the growth of *S. Aureus* (Haryadi, 2010). Amalia (2009) states that 20% betel leaf infusion is a solution that has the potential to be antiseptic.

Red betel contains phenol, which functions as an antiseptic. Phenols also denaturate proteins, so they have antimicrobial activity with phenol concentrations of 1-2%, 5% concentrations can cause tissue irritation (Katzung, Betram, 1998). According to Nisa, GK, et al (2014), the phenol content of red betel is 1.7-15%, so that it can be analogous to the phenol content of 20% red betel is 0.34 - 3.068% and for 40% content of 0.68 - 6%.

Wound washing is effective in removing dirt that can support bacterial growth, including the selection of cleansing techniques. Swabbing and irrigation are two types of cleaning techniques that are often used (Sussman & Bates, 2012). Irrigation is a more suggested method in cleaning wounds to eliminate bacteria (Crowley, Kanakaris, Giannoudis, 2007). Post hoc test results from the ANOVA cleansing technique of irrigation with 20% red betel leaf infusion, and swabbing with 20% red betel leaf infusion are effective in the decreasing total number of bacteria with diabetic ulcer isolates. This is supported by a systematic review study by the Joanna Briggs Institute, that irrigation with a strength of 13 psi compared to cleaning using gauze, does not show differences in the degree of infection of the 2 cleaning techniques. The opinion of Jones, ML (2012) states that swabbing with gauze or

cotton can remove debris from the wound surface, swabbing with cotton or gauze collected can remove slough and necrotic tissue. However, washing swabbing techniques can eliminate or damage tissue and epithelium due to trauma to the wound. This is in accordance with the opinion of Eissa MEA and Mahmoud AM (2012), that swabbing can clean bacteria up to 96% of the wound surface.

In this study the results from cleansing techniques with 20% red betel leaf infusion and swabbing using 20% red betel leaf infusion showed better results in decreasing number of bacteria than other results of treatment in this study, this when swabbing is used, the smears of gauze bind more bacteria. While irrigation techniques can be caused due to wounds, there are still hairs of test animals, which can cause bacteria that dissolve with irrigation fluids or get stuck in the hairs of test animals, so bacteria that not dead can return to the wound.

Wounds present in bacterial colonization as well as infections are also very likely biofilms, it is very difficult to detect the presence of biofilms in wounds, while biofilms are also very bother at repairing wounds. Swabbing is very possible to lift biofilms that can be a place for bacterial growth as well (Eissa MEA and Mahmoud AM, 2012; Swanson Terry et al, 2015).

Success in wound cleansing is determined by 3 aspects, which is cleansing techniques, and the right selection of tools and materials (Roberts, 2006). The results in this study the total bacterial numbers of diabetic isolates with cleansing intervention with swabbings technique generally showed smaller numbers compared to distribution techniques in all groups. Determination of the use of the technique and the type of solution can be adjusted to the wound

condition by considering the aspects of its advantages and disadvantages. The results of a study conducted by Mask (2014) which agreed to a comparison with swabbing, irrigation is more effective in terms of time, cost, comfort and patient satisfaction, for Swabbing techniques can also be used for wounds with biofilms that are difficult to remove.

An alternative recommended for cleansing is a swabbing technique using 20% red betel leaf infusion. Financial considerations in terms of financing and minimum doses in terms of pharmacology can also be taken into consideration in the application. According to Sussman (2014), the use of red betel leaf infusion solution (*Piper crocatum*) or antiseptic use can be used optimally for 14 days of treatment, to avoiding the toxicity and resistance. After 14 days of treatment, cleansing can use 0.9% normal saline solution.

CONCLUSION

Based on the results of research and discussion, it can be concluded that:

1. Cleansing with irrigation and swabbing techniques using 20% red betel leaf (*Piper crocatum*) infusion is effective in the total bacterial number of diabetic ulcer isolates in alloxan-induced white rats.

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