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The Effectiveness of Garlic Extract Ointment on the Healing of Perineal Wounds in Mice

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ABSTRACT

Perineal wound is one of the most frequently infected areas during the puerperium and is the second most common cause of primary postpartum hemorrhage. The allicin compound in garlic acts as an anti-platelet cell aggregation, anti-inflammatory and fibrinolysis booster that can accelerate the wound healing process. The aim of the study was to analyzing the effectiveness of 30% garlic extract ointment compared to 10% povidone iodine on perineal wound healing in rats. The research design is *true experiment* with the post test method with control group on 12 wistar rats. The wound was given as long as ±0.5 cm deep to the dermis in the perineal area of the rat. The control group was given 10% povidone iodine ointment and the intervention group was given 30% garlic extract ointment. The measuring instrument in this study used the REEDA scale and histological examination. Result of research showed that wound healing in the intervention group was faster than the control group (6 days versus 7 days). The results of independent t-test on the REEDA scale and histology examination showed sig. 0.000 and 0.0017 which means that there is a difference between the two groups based on the two measuring instruments. The use of 30% garlic extract ointment is more effective for healing perineal wounds.

Keywords:*Garlic Extract, Perineal Wound, Postpartum*

INTRODUCTION

Perineal wound is one of the areas most frequently affected by infection during the puerperium and is the second most common cause of primary postpartum hemorrhage after uterine atony. It is estimated that 1-8% of mothers will develop a postpartum infection.[1] The incidence of perineal injuries in the world is predicted to increase by 6.3 million cases in 2050. The number of perineal lacerations in Indonesia in 2013 was 57% from 1951 vaginal deliveries.[2] In accordance with midwifery care during the puerperium, it is necessary to periodically observe the perineal wound. The REEDA scale (redness, edema, ecchymosis, discharge, approximation) can be used to observe perineal wounds.[3] Another parameter to measure the wound healing process is the epithelialization process. Epithelialization measurement can be done by taking a sample of wound tissue and making histological preparations with HE (Hemaktosilin Eosin) staining which is then measured the thickness of the epithelium with the help of a microscope.[4]

Perineal care that is usually done is to give red medicine or treat with NaCl and let it dry. The use of red medicine causes uncomfortable pain and is easier to disappear when exposed to water.[5] Handling to reduce pain, which is done pharmacologically, has the potential to provide side effects such as the analgesic mefenamic acid which can cause pain in the mother's stomach.[6]

Garlic contains allin which is a derivative of one of the organosulfur compounds.[7] The amino acid allin can be turned into allicin by squeezing or chopping garlic. Allicin compounds act as anti-platelet cell aggregation, anti-inflammatory and fibrinolysis booster which can help accelerate the wound healing process.[8] Other phytochemicals contained in garlic are flavonoids which function as antioxidants. Antioxidants function to reduce inflammation in wounds.[9] Garlic is used as a topical preparation in the form of an ointment with a dose of 30%. The preparation of the ointment was chosen because the ointment can penetrate into the top layer of the skin so that it has a healing effect.[10]Making ointments based on fatty ointments (vaselin album and adeps lane) which have the property of attracting more water so that the wound dries quickly.[11] Healing of cuts shows better results by using fat-based ointments. The hydrocarbon compounds in Vaseline are able to moisturize the skin and are difficult to wash which makes it difficult for foreign objects to enter the wound so as to minimize the possibility of infection. Adeps lane has fluid absorption in the wound so that the wound dries quickly.[12]

In general, this study aimed to analyze the effectiveness of 30% garlic extract ointment compared to 10% povidone iodine on perineal wound healing in rats.

METHODS

This research is an analytical research with a true experimental research design and using a post test design with control group. This research has received permission from the ethics commission from the Medical/Health Research Bioethics Commission, Faculty of Medicine, Sultan Agung Islamic University, Semarang No. 39/II/2021/KomisiBioetik signed February 22, 2021. The subjects of the study were 12 female wistar white rats. Sampling using purposive sampling that is by adjusting the research criteria that have been determined. The inclusion criteria of the study were female wistar white rats, aged 16-20 weeks, body weight 200-250 gr, perineal wound length 0.4-0.6 centimeter, no anatomical abnormalities, healthy and active. The exclusion criteria for this study were rats were weak during treatment, rats refused to eat and rats died during treatment.

The treatment of rats was carried out at the Biology Laboratory of the State University of Semarang. The 12 rats that had been selected using the purposive sampling method were then separated into two groups, namely the control group which was treated with 10% povidone iodine ointment and the intervention group which was treated with 30% garlic extract ointment. All rats then went through an adaptation period with standard feeding in a new cage for 7 days. On the 8th day all rats were anesthetized using Ketamine at a dose of 0.3 ml/head and then given an incision in the perineal area of the rat. Wounds were assessed using the REEDA scale every morning for 7 consecutive days. Povidone iodine and garlic extract ointment were applied twice a day in the morning (after the REEDA scale assessment) and in the evening for 7 days. On the 8th day after the perineal incision was given, all rats were terminated using inhaled chloroform overdose. After termination, the tissue around the perineal wound was taken to be used as histological preparations. The wound tissue that was taken was then placed into 10% formalin solution. The preparation and examination of histology preparations was carried out at the Diponegoro National Hospital Laboratory, Semarang.

This study used two measuring instruments, namely the REEDA scale and histological examination. The REEDA scale assessment consists of redness (redness), swelling (edema), bleeding spots at the edges of the wound (ecchymosis), discharge (discharge) and approximation of the wound. Each has a score of 0-3 with an average REEDA score of 0-15 points. The lower REEDA score average points indicate better wound healing. Histological examination was carried out by measuring the thickness of the epithelial tissue in the wound. The thicker the epithelial tissue formed, the better the wound healing process. This is because in a healing wound, epithelial tissue will form from the base of the wound to the outer part of the wound and then it will close to form scar tissue.

Garlic extract was made by maceration method of 96% ethanol with the final result in the form of a thick extract. The basic ingredients of the ointment used were adaps lane and vaseline album as much as 50 grams with a composition of 7.5 grams adaps lane and 42.5 grams vasalin album. Garlic extract ointment was made as much as 50 grams by mixing 15 grams of thick garlic extract and 35 grams of the basic ingredients of the ointment. Garlic extract ointment has been tested for content, organoleptic test, homogeny test and pH test with the results that

this ointment is safe to use and does not cause allergies.

RESULTS

Twelve rats were placed into two study groups, 6 rats each. There were no rats that dropped out until the end of the treatment.

Table 1 :Frequency distribution from REEDA Scale and histological measurement based on demographic data

Measuring Instrument	Group	N	Mean	SD	Min	Max
REEDA Scale	Intervention	6	32,50	1,52	30	34
	Control	6	41,33	5,43	35	50
Histological measurement	Intervention	6	36,00	16,72	22	61
	Control	6	26,83	12,04	13	44

The results of the REEDA scale measurement have an interpretation that the lower the results, the better the wound healing. The measurement results showed that the average in the intervention group had a lower value than the control group, which means that the intervention group had better wound healing than the control group. The mean and standard deviation of the control group with histological examination measuring instruments showed 26.83±12.04 results and the intervention group was 36.00±16.72. The results of the histological examination have an interpretation that the thicker the size of the epithelial tissue, the better the wound healing. The measurement results showed that the average in the intervention group had a higher value than the control group, which means that the intervention group had better wound healing than the control group.

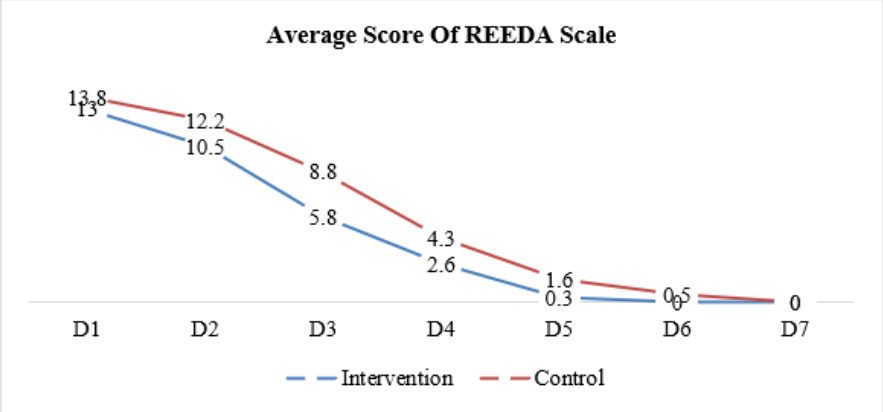


Chart 1: Average Score of REEDA Scale

The chart above shows that the average REEDA score in the intervention group is lower than the control group. A lower average REEDA score indicates better wound condition. From this graph, it can be concluded that wound healing in the intervention group (30% garlic extract ointment) was better than the control group (10% povidone iodine). The results of the repeated measures test showed a significance value of 0.000 which means that there is a difference in the average decrease in the results of the REEDA scale in the control group and the intervention group.

Table 2 Analysis of the differences in mean REEDA scale and histological examination between the intervention group and control group

Measuring instrument	Group	Total Value	homogeneous p-value	Mean	SD	P-value
REEDA Scale	Intervention	195	0.083	32.50	1.52	0.003
	Control	248		41.33	5.43	
Histological Examination	Intervention	2.16 mm	0.331	36.00	16.72	0.017

	Control	1.61 mm	26.83	12.04

**Independent t test*

The table above shows the results that the Independent T-Test for the control and intervention groups using the REEDA scale has a significance value of 0.003. The results of the Independent T-Test for the control and intervention groups with histological examination measuring instruments had a significance value of 0.017. The significance value of the two groups using two measuring instruments showed results <0.05. The interpretation that can be taken is, Ho is rejected and Ha is accepted or there are differences in the results of the REEDA score and the results of histology examination in the 10% povidone iodine control group and the 30% garlic extract ointment intervention group.

DISCUSSION

The healing process of injured skin is a complex process that requires the collaborative effort of many different skin tissues to repair and regenerate injured skin tissue. Wound healing mechanisms have developed in the past few years, wound healing studies have provided better developments both exogenously and by pathological factors which can actually lead to abnormal wound healing.[13] Several studies have shown that garlic extract can improve the wound healing process and can reduce the risk of infection in the wound. Fibrinogen is activated by allicin present in garlic and it is known that fibrinogen is the most important element in the wound healing process. Garlic in ointment preparations has the best healing potential.[14]

Various innovations have been investigated both promotive in the form of educationand curative in the form of treatment. In this research, a new innovation is made by using garlic extract made in ointment preparations.[15] The ointment mixture used is fat-based, such as white albumin vaseline and adaps lane which can accelerate wound drying.[12] The difference between this study and other studies is the type of wound studied, namely the perineal incision wound which is an area that is easily infected and has a thin skin thickness. A thin layer of skin is known to have more blood vessels so it is easy to bleed and there is less collagen, whereas in the proliferative phase collagen is needed to accelerate wound healing.[16]

The most important component in the tissue healing process is the rearrangement of the collagen network which can affect the quality of the wound. The mechanism of tissue arrangement that occurs during the wound healing process is not clearly known. There are two theories that can explain this mechanism, one of it because there is a rearrangement of the collagen network due to the activity of fibroblasts.[13] The results of this study are the same as the theory above, the results of histological examination of the perineal wound showed that the thickness of the epithelial tissue of the perineal wound in the intervention group was better. Epithelial tissue can cover and regenerate better because of the allicin content in garlic which functions as a fibrinolysis booster so that it can increase collagen activity in wound tissue.[8]

The use of herbal plants is often used as an alternative to overcome various obstetric problem,[17] include the use of herbal plants as an alternative to perineal wound healing interventions. Research by NajmehBabadi (2018) in Iran used sesame seed extract ointment to accelerate the healing of perineal wounds. Sesame seeds are known to have anti-inflammatory, anti-bacterial and anti-oxidant activities.[18] The results of this study showed that there were differences in the results of the REEDA scale of perineal wounds on the 7th and 10th days. Perineal wound healing began to show since the 7th day and got better on the 10th day.[18] In this study, perineal wounds can heal faster, namely on the 6th day. Histological examination results also showed that the perineal wound regeneration in the intervention group was better than the control group. Epithelial tissue can cover and regenerate better because of the allicin content in garlic which functions as a fibrinolysis booster so that it can increase collagen activity and can increase fitroblast tissue in wounds. Not all herbal plants have the ability to stimulate fibrinolysis in wounds, therefore garlic can heal wounds faster than some of the herbs above. It has been explained that there are two mechanisms of wound healing, one theory is that wounds can heal because of fibroblast activity which can increase collagen tissue in wounds so that wounds can heal quickly (Hernawan and Setyawan, 2014).

Garlic bulbs also have anti-inflammatory activity to stop inflammation and anti-platelet cell aggregation to help collagen formation. A journal review by Hernawan mentions the benefits of garlic as an anti-platelet cell aggregation which can then help the formation of collagen to accelerate the closure of open wounds.[8] The results of this study are in line with the journal, it is known that garlic extract ointment has more total thickness of epithelial tissue than povidone iodine ointment. Garlic extract ointment can help speed up the wound closure process so that it has a shorter healing time and can minimize the occurrence of infections caused by the entry of microorganisms

into the wound. Infection in the perineal wound is often a problem during the puerperium and is known as the number two cause of maternal death during the puerperium.[1] This can be avoided by applying garlic extract ointment. Microbiological test of garlic extract showed that this extract could inhibit the growth of various microorganisms including bacteria, protozoa, viruses and fungi.[8, 19] The ointment preparation used in this study also contributed to preventing the entry of microorganisms that cause wound infections. The hydrocarbon compounds in Vaseline are able to moisturize the skin and are difficult to wash, which makes it difficult for foreign objects to enter the wound, thereby minimizing the possibility of infection.[12]

This study shows that 30% garlic extract ointment can be used as an alternative to heal perineal wounds in rats compared to povidone iodine ointment which is generally used in wound care. The measurement results and test results in this study have answered the problem formulation, objectives and existing hypotheses. The advantage of this study compared to other studies is that there is a new innovation for topical preparations of garlic extract where not all extracts can be homogeneous with ointment base ingredients and in this study garlic extract was proven to be homogeneously mixed with ointment base ingredients. Another advantage is that it is triple blinded where subjects, researchers and assessors do not know which group is given the ointment and povidone iodine. The limitations of this study are that the perineal wound should always be kept clean and dry, the care that should be used is bathing or rinsing the perineal wound with the intervention to be used. In this study, the treatment by soaking or rinsing could not be used because of the limitations of the tools in the laboratory used.

CONCLUSION

The application of 30% garlic extract ointment and 10% povidone iodine was effective for healing perineal wounds. The application of 30% garlic extract ointment has a faster healing time and better wound results than 10% povidone iodine ointment.

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