Original Research Article

Test Betel Leaf *(Piper betle L.)* Extract For Wound Healing In White Rats

ABSTRACT

The use of natural ingredients as a treatment and wound healing has been widely used, one of which is betel leaf (*Piper betle Linn*). This study aims to analyze and test whether betel leaf extract can accelerate wound healing in male white rats (Rattus norvegicus). This study used an experimental research design. The sample in this study were male white rats grouped randomly into 4 groups with each consisting of 5 rats, then given a 2 cm incision wound and given different treatments. The first group was the treatment groups with 2.5% betel leaf extract cream. The second group was given 5% betel leaf extract cream. Third groups with 10% betel leaf extract cream. The fourth group was the control group with NaCl 0.9%. The length of the wound in each group was observed and measured every day for 14 days. The data obtained is then analyzed using One Way ANOVA. The result showed that the wound healing process between treatment groups differed significantly because the level of effectiveness and optimization of the doses also varied, namely the sequence of treatment groups that accelerated the wound healing process was the betel leaf extract cream 10%, 5%, 2.5% and last NaCl 0.9%. Thus, it was concluded that the giving of betel leaf extract cream can accelerate the healing of wounds in male white rats which are characterized by the higher dosage of betel leaf extract will further accelerate the healing of incisions in male white rats.

Keywords: Betel Leaf Extract Cream; Wound Healing

1. INTRODUCTION

Wounds are damage to the continuity of the skin, mucosa, and bones or other organs that can occur accidentally and intentionally for certain purposes. A wound is damage to a part of the body that occurs on the skin which is a tissue that is cut off, torn, or damaged by a cause (Librianty, 2015). Wounds are also lost or damaged in part of the body tissue. This situation can be caused by trauma, sharp or blunt objects, changes in temperature, chemicals, or animal bites. Wounds cannot be allowed to heal on their own because wounds cannot be proven can cause wound complications can occur infection and bleeding. The purpose of treating wounds is to prevent trauma (injury) to the skin, mucous membranes or other tissues caused by trauma, fractures, surgical wounds that can damage the surface of the skin.

In traumatology there are several categories of injuries, including categories of injuries based on their causes, such as cuts, bruises, stab wounds, abrasions, and torn wounds (Wombeogo & Kuubire, 2014). Incisions are usually caused by sharp objects such as knives, razors, or scalpels in the surgical process characterized by the edges of the wound in the form of straight and regular lines (Wombeogo & Kuubire, 2014). The wound healing process is important because the skin is a single organ that is exposed to the outside world. The skin has specific functions for the body, namely protective, sensory, thermoregulatory, metabolic, and sexual signals. When the skin loses continuity, these functions cannot work as they should (Mescher, 2012). Therefore, the wound healing process requires proper management and treatment so that the wound area does not become infected and eventually causes chronic wounds.

The wound healing process is a physiological process that involves several components which include cells and chemical substances needed in the inflammatory process, angiogenesis, and collagen deposition (Sorg, et. al., 2017). This process goes through several phases, namely hemostasis, inflammation, proliferation, and remodeling. The hemostasis phase occurs shortly after the wound which is characterized by the formation of platelet aggregation. This process is needed to cover damage to blood vessels (Mori, et. al., 2008). The next phase is inflammation occurring 1–4 days after the wound. This phase is characterized by infiltration of neutrophil cells and macrophages in wound tissue. Macrophage cells will emit inflammatory mediators and enzymes to start

the next phase, namely the proliferation phase. The proliferation phase occurs 4 to 21 days after injury, characterized by angiogenesis, collagen deposition, granuloma tissue formation, wound contraction, and epithelialization. The last phase is remodeling which occurs 21 days to 2 years after a wound occurs. This phase is characterized by the formation of new tissue that has been intact (Rohl, et. al., 2015).

The wound healing process is influenced by both local and systemic factors, one of which is wound care (Rohl, et. al., 2015). Good wound care will accelerate the process of wound healing and also good tissue formation. Handling wounds that can be done in the form of antiseptics, antibiotics, and wound care in general. Treatment can be done by giving drugs that are commercial in nature or in the form of alternatives using ingredients found in nature. The use of natural ingredients as a treatment and wound healing has been widely used, one of which is betel leaf (Piper betle Linn). Traditionally betel leaf is used as a medicine for thrush, sore throat, cough medicine, eyewash medicine, vaginal discharge, stop bleeding in the nose or nosebleeds, accelerate wound healing, and eliminate bad breath. Pharmacologically, betel leaves have styptic properties (resist bleeding), stomachic (digestive tract medicine), cure skin wounds, astringent, diuretic, and anti-inflammatory.

Betel leaves contain saponins, flavonoids, tannins and essential oils. The saponin content, flavonoids and tannins can help the wound healing process because it functions as an antioxidant and antimicrobial which affects the connection of wounds and also accelerates epithelialization (Saroja, et. al., 2012). The content of saponins and tannins play a role in tissue regeneration in the process of wound healing (Reddy, et. al., 2011). The saponin content has the ability as a cleanser or antiseptic. Saponins can trigger vascular endothelial growth factor (VEGF) and increase the number of macrophages migrating to the wound area thereby increasing the production of cytokines which will activate fibroblasts in wound tissue (Kimura, et. al., 2006). The content of flavonoids functions as an antioxidant, antimicrobial and also anti-inflammatory in burns (Park, et. al., 2010). The onset of cell necrosis is reduced by flavonoids by reducing lipid peroxidation. Inhibition of lipid peroxidation can increase the viability of collagen fibers, blood circulation, prevent cell damage and increase DNA synthesis. The content of tannins has astringent, antioxidant and antibacterial abilities (Park, et. al., 2010). The content of tannins accelerates wound healing with several cellular mechanisms, namely cleaning up free radicals and reactive oxygen, increasing splicing of wounds and increasing the formation of capillary blood vessels as well as fibroblasts (Sheikh, et. al., 2011). While essential oils contain kavikol and phenol which are useful as antimicrobial, antibacterial and disinfectant (Park, et. al., 2010).

Based on the descriptions above, betel leaf can function as an antiseptic, antioxidant, antimicrobial, antibacterial, anti-inflammatory, and astringent so that it can accelerate the wound healing process. The benefits of a large betel leaf in wound healing is an important reason for researchers to study more deeply by conducting experimental research on the test of betel leaf extract (piper betle L.) to accelerate wound healing in male white rats (Rattus norvegicus). The purpose of this study was to analyze and test whether betel leaf extract (piper betle L.) can accelerate wound healing in male white rats (Rattus norvegicus).

2. MATERIALS AND METHODS

This study included laboratory experimental studies aimed at analyzing and testing the ability of betel leaf extract to accelerate wound healing in male white rats. The study was conducted in April 2019 at the Chemical Laboratory and Biology Laboratory, Faculty of Mathematics and Natural Sciences, Medan State University.

The samples in this study were adult male rats (Rattus norvegicus) weighing 160-200 grams and aged 2-3 months. The selection of experimental white rats is based on the consideration that it is most often used in biomedical research, a relatively large size compared to mice and has adaptability in a laboratory environment. The number of samples of white rats used in this study were 20 and were considered to be a large sample divided into 4 (four) groups with each group as many as 5, namely group A given betel leaf extract 2.5% (dose I), group B was given 5% betel leaf extract (dose II), group C was given 10% betel leaf extract (dose III) and the control group was only given 0.9% NaCl.

TABLE 1. TOPICAL DOSAGE FORMULATIONS FOR EACH CREAM

Material	Concentration (%)		
	F1	F2	F3
Betel leaf extract	**2,5**	**5**	**10**
Cetyl alcohol	4	4	4
Glycerin	15	15	15
TEA (triethanolamin)	3	3	3
Stearic Acid	12	12	12

Metylparaben	0,2	0,2	0,2
Prophylparaben	0,02	0,02	0,02
Aquadest	100	100	100

Treatment of incisions in these mice was carried out twice a day, namely in the morning and evening for 14 days. Wound healing in white mice was observed by measuring the average wound length every day starting from the first day of wound making until the 14th day by calculating the percentage of wound healing using the formula:

$$P = \frac{do - dx}{do} \times 100\,\% \qquad (1)$$

Data from the results of subsequent studies were analyzed by normality test data using the Kolmogorov Smirnov or Shapiro-Wilk test (p> 0.05). One Way ANOVA test (p <0.05) to determine significant differences between the trial groups. Post-Hoc Homogeneous Tukey Subset test to find out which treatment group was the most significant among the trial groups.

3. RESULTS AND DISCUSSION

3.1 Description Of Research

This study used green betel leaf extract made in a cream at a concentration of 2.5%; 5% and 10% are expected to accelerate wound healing in male white mice. The male white rats used were 20 with a weight of 160-200 grams. The treatment of incisions in mice is done by applying a thin cream of betel leaf extract to the wounds of white rats 2 times a day, morning and evening and observed every day for 14 days.

TABLE 2. AVERAGE WOUND HEALING PERCENTAGE

Days	Average Wound Healing Percentage (%)			
	NaCl 0,9%	EDS 2,5%	EDS 5%	EDS 10%
0	0,0	0,0	0,0	0,0
1	2,0	3,0	4,0	12,0
2	7,0	12,0	9,0	31,0
3	12,0	18,0	20,0	37,0
4	17,0	26,0	30,0	49,0
5	21,0	33,0	38,0	62,0
6	26,0	41,0	45,0	69,0
7	32,0	50,0	53,0	74,0
8	40,0	58,0	61,0	81,0
9	49,0	65,0	72,0	87,0
10	56,0	74,0	78,0	92,0
11	64,0	84,0	91,0	96,0
12	70,0	93,0	100	100
13	78,0	100	100	100
14	85,0	100	100	100

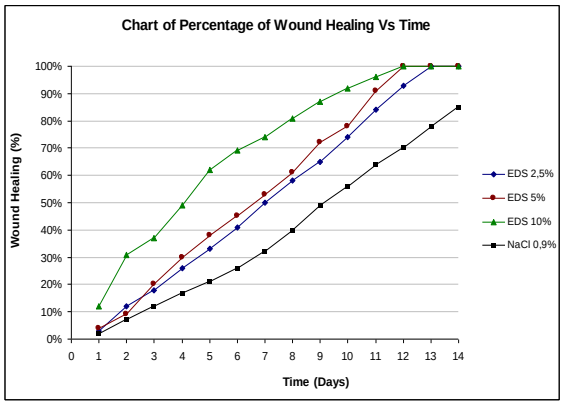

Figure 1: Chart of Percentage of Wound Healing

Vs Time

Based on the graph in Figure 1, shows the average wound healing process in male rats (100% wound closure) occurred on the 12th day for the treatment group given 5% and 10% betel leaf extract cream (5% EDS and 10% EDS) For the treatment group given 2.5% betel leaf extract cream (EDS 2.5%) the average 100% wound healing process occurred on the 13th day. While for the control group given 0.9% NaCl the wound closure process only reached an average of 85%.

3.2 Data Analysis

To test whether betel leaf extract can accelerate wound healing in white rats, data analysis was carried out with the help of the SPSS program.

TABLE 3. *One Way ANOVA*

ANOVA

Wound Length

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13.410	3	4.470	12.096	.000
Within Groups	101.994	276	.370		
Total	115.404	279			

Based on the results of data analysis in Table 2 above, the value of F-count is 12,096 with a significance value or probability (p) <0.05. This indicates that there is a difference between the groups given 2.5%, 5% and 10% betel leaf extract cream and those given 0.9% NaCl in accelerating the wound healing process in male white rats. Thus it was concluded that betel leaf extract (piper betle L.) can accelerate wound healing in male white rats.

Table 4: *Homogeneous Subsets*

	Percentage	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	EDS 10%	70	.586		
	EDS 5%	70		.856	
	EDS 2,5%	70		.919	
	NaCl 0,9%	70			1.201
	Sig.		1.000	.928	1.000
Duncan[a]	EDS 10%	70	.586		
	EDS 5%	70		.856	
	EDS 2,5%	70		.919	
	NaCl 0,9%	70			1.201
	Sig.		1.000	.541	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 70.000.

The results of the Tukey test and the Duncan test in Table 3 above, showed that the treatment group using 10% betel leaf extract cream (10% EDS) differed significantly compared to the other treatment groups. For the treatment group using betel leaf extract cream 2.5% and 5% (2.5% EDS and 5% EDS) did not differ significantly. While for the control group (NaCl 0.9%) it was significantly different than the other groups.

Based on the results of the analysis above, it can be concluded that the administration of 10% betel leaf extract (EDS 10%) is better in accelerating the wound healing process in male white rats compared to other treatment groups. While the administration of 2.5% betel leaf extract (EDS 2.5%) and 5% (EDS 5%) is equally good in

accelerating the wound healing process in male white rats and better than the control group without cream of betel leaf extract (NaCl 0.9%).

The results of the research conducted are in line or support the results of previous studies conducted by Latuheru, et al. (2013), about the effect of betel leaf (Piper betle L.) on rabbit skin incision healing (Oryctolagus cuniculus). The conclusion of the study is that the administration of betel leaves has an effect to accelerate healing of rabbit skin incision wounds. The results of other studies were conducted by Fannani and Nugroho (2014), about the effect of ointment of betel leaf ethanol extract (Piper betle) on healing iris wounds in male white mice (Rattus norvegicus). The study used 30 rats grouped into 3 groups with each group consisting of 10 rats, then injured 2 cm deep 0.5 cm deep and given different treatments. The results of the study concluded that the betel leaf extract of ethanol was able to accelerate the healing process of iris wounds in male white rats.
Pharmacologically, betel leaves have styptic properties (resist bleeding), stomachic (digestive tract medicine), cure skin wounds, astringent, diuretic, and anti-inflammatory. Betel leaves contain saponins, flavonoids, tannins and essential oils. The saponin content, flavonoids and tannins can help the wound healing process because it functions as an antioxidant and antimicrobial which affects the connection of wounds and also accelerates epithelialization (Saroja, et. al., 2012). The content of saponins and tannins play a role in tissue regeneration in the process of wound healing (Reddy, et. al., 2011). The saponin content has the ability as a cleanser or antiseptic. The content of flavonoids functions as an antioxidant, antimicrobial and also anti-inflammatory in burns (Park, et. al., 2010). The content of tannins has astringent, antioxidant and antibacterial abilities (Park, et. al., 2010). The content of tannins accelerates wound healing with several cellular mechanisms, namely cleaning up free radicals and reactive oxygen, increasing splicing of wounds and increasing the formation of capillary blood vessels as well as fibroblasts (Sheikh, et. al., 2011). While essential oils contain kavikol and phenol which are useful as antimicrobial, antibacterial and disinfectant (Park, et. al., 2010). All content of betel leaves can clean the wound and prevent infection so that it can accelerate the end of the inflammatory phase in the process of wound healing.

4. CONCLUSIONS

Based on the results of research and experiments conducted it can be concluded that the administration of betel leaf extract (piper betle L.) can accelerate the healing of wounds in male white rats which are characterized by higher doses of betel leaf extract will accelerate the healing of wounds in male white rats. The wound healing process between treatment groups was significantly different because the level of effectiveness and optimization of the doses also varied, namely in the order of treatment groups that obtained a 100% wound healing process (100% wound closure) is 10% betel leaf extract cream (starting on the day -9 or average on day 12), 5% (starting on day 11 or average on day 12), 2.5% (starting at day 11 or average on day 13th) and finally 0.9% NaCl group (on the 14th day wound closure only reached 85%).

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

REFERENCES

1. Fannani, M.Z., dan Nugroho, T. 2014. Pengaruh Salep Ekstrak Etanol Daun Sirih (Piper betle) terhadap Penyembuhan Luka Iris Pada Tikus Putih Jantan (Rattus norvegicus). *JKKI*. 6(1): 19-26
2. Kimura, Y., Sumiyoshi, M., Kawahira, K., and Sakanaka, M. 2006. Effects of Ginseng Saponins Isolated from Red Ginseng Roots on Burn Wound Healing in Mice. *British Journal of Pharmacology*. 148: 860-870.
3. Latuheru, J.O., Tambajong, J.W., dan Posangi, J. 2013. Efek Daun Sirih (Piper Betle L.) terhadap Penyembuhan Luka Insisi Kulit Kelinci (Oryctolagus cuniculus). *Jurnal e-Biomedik (eBM)*. 1(2): 802-805.
4. Librianty, N. 2015. *Panduan Mandiri Melacak Penyakit*. Jakarta: Lintas Kata.
5. Mescher, A.L. 2012. *Histologi Dasar Junqueira: Teks & Atlas*. Jakarta: EGC.
6. Mori, R., Shaw, T.J., and Martin, P. 2008. Molecular Mechanism Linking Wound Inflammation and Fibrosis: Knockdown of Osteopontin Leads to Rapid Repair and Reduced Scarring. *J Exp Med*. 205(1): 43–51.
7. Park, *et. al*. 2010. Protection of Burn-Induced Skin Injuries by the Flavonoid Kaempferol. *BMB Reports*. 43(1): 46-51.

8. Pradhan, D., Suri, K.A., Pradhan, D.K., and Biswasroy, P. 2013. Golden Heart of Nature: Piper betle L. *Journal of Pharmacognosy and Phytochemistry*. 1(6): 147-167.
9. Reddy, B.K., Gowda, S., and Arora, A.K. 2011. Study of Wound Healing Activity of Aqueous and Alcoholic Bark Extracts of Acacia catechu on Rats. *RGUHS Jour-nal of Pharmaceutical Sciences*. 1(3): 220-225.
10. Rodero, M.P. & Khosrotehrani, K. 2010. Skin Wound Healing Modulation by Macrophages. Int. *J. Clin. Exp. Pathol*. 3(7): 643–653.
11. Röhl, J., Zaharia, A., Rudolph, M., and Murray, R.Z. 2015. The Role of Inflammation in Cutaneous Repair. *Wound Pract Res*. 23(1): 8-15.
12. Saroja, M., Santhi, R., and Annapoorani, S. 2012. Wound Healing Activity of Flavonoid Fraction of Cynodon dactylon in Swiss Albino Mice. *International Research Journal of Pharmacy*. 3(2): 230-231.
13. Sheikh, AA., Sayyed, Z., Siddiqui, A.R., Pratapwar, A.S., and Sheakh, S.S. 2011. Wound Healing Activity of *Sesbania grandiflora Linn* Flower Ethanolic Extract Using Ex-cision and Incision Wound Model in Wistar Rats. *International Journal of PharmTech Research*. 3(2): 895-898.
14. Sorg, H., Tilkorn, D.J., Hager, S., Hauser, J., and Mirastschijski, U. 2017. Skin Wound Healing: an Update on the Current Knowledge and Concepts. *Eur Surg Res*. 58(1–2): 81–94.
15. Wombeogo, M. & Kuubire, C.B. 2014. *Trauma and Emergency Health Care Manual.* Bloomington: AuthorHouseTM UK Ltd.