Original article

Potential Healing Effect Of Mulberry Leaf Extract Oinment on Burn Injury in rats

Kamilia Dwi Utami[1], Ulfah Dian Indrayani[2]

Abstract:

Objective: Mulberry leaf contains Vitamin C, Flavonoids, Oxyresveratrol and Saponin, which accelerated reepithelialization and increases wound contraction in burn healing. This study was designed to asses the effect of mulberry leaf extract ointment (Morus Alba L) on number of fibroblast and collagen fraction area in second degree burns. ***Materials and methods:*** This was an experimental design for 6 days with 24 male in Sprague dawley rat randomly selected and divided into 4 groups. The back of Sprague dawley male rats were shaved and given burns using 4 x 2cm iron plate which was put in 98^{o} boiling water for 5 minutes and pasted for 10 seconds and smeared with distilled water, and ointment of mulberry leaf extract. Group I was a positive control (silver sulfadiazide ointment), Group II was a negative control (ointment base), Group III (mulberry leaf extract ointment 10%) and Group IV (mulberry leaf extract ointment 20%). The number of fibroblasts and collagen was evaluated with staining of hematoxylin eosin and staining mallory trichrome. ***Result and discussion:*** The mean of fibroblasts I, II, III,and IV was 21.33 ± 1.74, 13.28 ± 1.48, 17.56 ± 1.75, and 23.11 ± 2.38, respectively. The mean collagen fraction area I, II, III, and IV was 21, 11±7,42 , 16,18 ±7,72, 26,078 ± 8, 00 , and 25,79±8,33 respectively. One way Anova followed LSD test showed significant different in mean of between each treatment group obtained significant differences ($p < 0.05$). The mean number of fibroblast in the 10% and 20% ointment extract were significantly different compared with negative controls. Collagen fraction area in treatment group with 10 and 20% ointment were significantly different from that of negative control group, and was not significantly different from silver sulfadiazine group ***Conclusion:*** The mulberry leaf extract can be an alternative treatment for second degree burns

Keyword: Mulbery leaves extract oinment, fibroblast, collagen, burns

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Introduction

Burns is one of the most common causes of pain and death in the world. WHO data showed that as many as 265,000 people died every year due to burns. Commonly used therapy standard for burns treatment is silver sulfadiazine ointment. Several researches showed that silver sulfadiazine has negative effects such as hypersensitivity reaction, Steven-Jonson syndrome and toxic epydermalinecrolysis and toxic to fibroblasts. Meanwhile, fibroblasts plays a major role in wound healing reconstruction phase in producing collagen, elastin, bicarbonic acid, fibronectin, and proteoglican[1]

Alternative therapy of silver sulfadiazine that has been studied is mulberry leaves (*Morus alba* L.). Former research about mulberry leaves extract showed 20% mulberry leaves cream able to accelerate the healing of second-degree burn wound. It was indicated by re-epithelialization and increasing of wound contraction[2]. However, there is no research yet about the effect of mulberry (*Morus alba* L.) leaves extract based cream to the percentage of collagen in the second-degree burn wound healing.

Flavonoid on mulberry leaves can reduces the inflammatory process by inhibiting prostaglandins that formed by arachidonic acid and other inflammatory mediators such as histamine and serotonin. It is because they are selective COX-

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2 inhibitor. This inhibition causes the increasing migration of neutrophils (acute inflammatory cells) and monocytes (chronic inflammatory cells) to the wound area. In the end of the inflammatory stage, macrophages extract TGF-β, FGF so it can increase the migration and proliferation of fibroblast cells[3,4]. Vitamin C is an antioxidant that will neutralize free radicals, bacterial phagocytosis products, and debris in wound healing process, and also stimulates collagen biosynthesis, and increase immunity. Oxyresveratrol compound plays a role as an anti-inflammatory agent, so it can accelerate fibroblasts migration. Saponin in mulberry leaves will affect FGF so it can increase the migration and proliferation of fibroblast that will from collagen.

A research by Bathia[5] in 11 days was the continuation of the former research. It combined 20% mulberry leaves extract and 20% neem leaves extract in the second-degree burn wound showed that there was nearly complete re-epithelium and re-structure and wound tissue[5]. Mulberry leaves contain several chemical compounds such flavonoid, oxyresveratrol, saponin and vitamin C that useful to increase the quantity of fibroblast. This fibroblast is a connective tissue that forms collagen fiber to contribute the strength and integrity of wound so it can heal properly [2]. Based on the description of mulberry leaves (*Morus alba* L.) In the above background, so it is required to study the effect of mulberry leaves (*Morus alba* L.) extract ointment to the percentage of collagen in the second-degree burn wound healing process in *Sprague dawley* rat skin.

METHODS

The type of this research was an experimental study by using "post-test only randomized controlled group design" research design. The independent variable of this study was mulberry leaves extract while dependent variable assessed was the percentage of collagen area fraction. Mulberry leaves extract was an ointment made of 1 kilogram of mature dark green mulberry leaves extracted by ethanol 96% 1.6 liter using maceration method to produce 6 gram extract and then mixed with ointment base. Mulberry leaves extract ointment was then made in several different doses, namely 10% dose (0.5 gram of mulberry leaves extract and 4.5 gram of ointment base), 20% dose (1 gram of mulberry leaves extract and 4 gram of ointment base) and 40% dose (2 gram of mulberry leaves extract and 3 gram of ointment base). The percentage of collagen fraction area was calculated by preparing histopathological sample of skin section with burn wound about 1x1x0.01 cm taken on the 6th day after mulberry leaves ointment treatment. All samples were made of paraffin preparations stocks. The figure of collagen fiber is bluish green. It was colored with *Masson's Trichrome* and then measured under light microscope with magnification 100x in 5 fields of view. The percentage of collagen density was examined by using Image J fraction area method. The quantity of fibroblast was colored by using Hematoxylin eosin and then measured by using light microscope and optilab with Image J.

The test subject was male Sprague Dawley rat taken from the Laboratory of Mathematics and Natural Sciences Faculty of Universitas Negeri Semarang/ UNNES and kept at the Biology Laboratory of Medicine Faculty of Universitas Islam Sultan Agung (UNISSULA). The study subject was the population of research that met the inclusion criteria such as healthy, active rats, 2-3 months old, body weight 150-250 gram, no anatomical abnormalities and never been used for previous research. As many as 24 male *Sprague dawley* rats were divided into 4 groups, each group consist of 6 samples that randomly taken. Ointment was made at the Pharmacology Laboratory Faculty of Medicine Universitas Islam Sultan Agung (UNISSULA). The research was conducted at the Biology Laboratory Faculty of Medicine Universitas Islam Sultan Agung (UNISSULA).

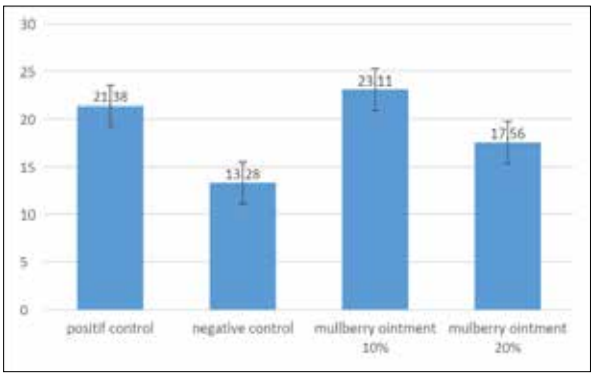
Data were tested using descriptive analysis to obtain mean, median, and standard deviation. *Shapiro-Wilk* test was used to identify normal and abnormal distribution of data and Levene's Test was used to identify the homogeneity of data. The results of Shapiro-Wilk test and Levene's Test were used as the parametric test requirement in the One Way Anova and then continued with Post hoc LSD test.

Ethical approval: The study was approved by the Ethical Committee Faculty of MedicineUniversitas Islam Sultan Agung, Semarang, Indonesia.

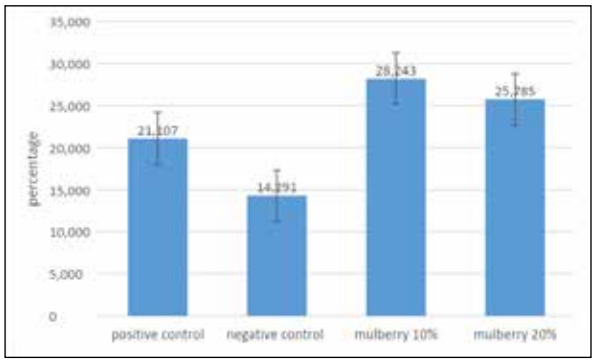
RESULT AND DISCUSSION

The group given 10% mulberry oinment had the highest amount of fibroblasts and the percentage of collagen area fraction compared to other groups. The average amount of fibroblasts and the percentage of collagen in each group are presented in the following figure:

The average quantity of fibroblast in the positive control group of 10% mulberry ointment and 20% mulberry ointment was significantly different compared to the negative control group. The



a. Amount of Fibroblast, p<0.05



a. Percentage of Collagen Area Fraction, p < 0.05

average quantity of fibroblast in the 20% mulberry ointment group was higher than 10% mulberry ointment group.

The average percentage of collagen in 10% and 20% group was significantly different with negative control group. The average percentage of collagen in 10% group was higher than 20% group.

DISCUSSION

The average fibroblast quantity in positive control group (silver sulfadiazine), 10% and 20% mulberry leaves (*Morus alba L*) extract ointment group showed significant difference if compared with negative control group. It showed that mulberry leaves extract ointment treatment affect to the increasing of fibroblast quantity in the rat skin with second-degree burn wound

The average percentage of collagen in 10% and 20% mulberry leaves (*Morus alba L*) extract ointment group was significantly different with the negative control. Therefore, it can be concluded that 10% and 20% mulberry ointment affect the synthesis of collagen.

This study was in line with the research results by Bathia[2]that mulberry leaves extract with 20% concentration in a period of 11 days given to second-degree burn wound was more effective to accelerate the speed of epithelialization and increase wound contraction compared to silver sulfadiazine in the rat skin[2].

The increasing of this fibroblast and collagen quantity was possibly caused by the content of mulberry leaves extract such as Vitamin C, flavonoid, tannins, alkaloid, saponin and phenol compounds that play a role in the collagen formation process. These chemical substances are flavonoid, tannins, alkaloid, saponin and phenol compounds. In addition, there are also vitamin A, beta carotene, vitamin C, catalase and quersitin as the derivatives of flavonoid glycosides. These natural ingredients can regenerate the damaged tissue with a variety of unclear mechanism [2].

Vitamin C is a water-soluble vitamin that can be used as antioxidant that will neutralize free radicals of bacterial phagocytic products and debris in the wound healing process [6]. Catalase is one of the enzymes that play a role in the breakdown of Hydrogen Peroxidase (H_2O_2) into water (H_2O) and Oxygen (O_2). Catalase in this decomposition process will reduce the danger of damage that caused by ROS [7]. In addition, vitamin C also stimulates the biosynthesis of collagen, carnitine, and neurotransmitter [8].

Flavonoid has main effects as the antioxidants and anti-inflammation. Antioxidants work to prevent free radicals. Antioxidants will bind to the outer electrons of free radicals. Free radicals have unstable outermost electrons because of their unpaired characteristic. Electron instability can damage cell membranes; disturb DNA metabolism, cells, and lipids. Bonded antioxidants can cause stable free radicals so the damage of cell membrane can be reduced. Flavonoid can reduce excessive inflammatory process by inhibiting prostaglandin that produced by arachidonic acid and other inflammatory mediators such as histamine and serotonin because they are selective COX-2 inhibitor. This inhibition can cause neutrophils migration (acute inflammatory cells) and monocytes (chronic inflammatory cells) to the wound area. In the end of the inflammatory stage, macrophages extract TGF-β, FGF so it can increase the migration and proliferation of fibroblast cells [3,4]. Non-prolonged inflammatory stage can cause proliferation stage faster and increase fibroblast so it can increase collagen synthesis.

Flavonoid also can increase the interaction process of cell and adhesion of molecules that play a very significant role in the proliferation phase and epithelialization phase to the wound tissue healing process. In addition, flavonoid can prevent or

prolong the onset of cell death especially fibroblast along with the increasing of vascularization of the wound. If fibroblasts are protected, fibroblasts can migrate to the wound area and there will be attachment between collagen and fibroblast in the wound edge so epithelium can thicken, especially on days 6-14. In addition, flavonoid also responsible to the increasing of wound contraction and epithelialization.

Saponin in wound healing plays its role in stimulating the formation of collagen that significant in the wound closing process and increase the epithelialization of tissue. Collagen is a structural protein that plays its role in the healing process. Saponin is able to stimulate the formation of new cells or known as growth factor, so it can cause multiplication and growth of blood vessel, endothelial cells, smooth muscle cells of blood vessels and fibroblasts so it can lead to cellular growth that finally repairs the damaged blood vessel walls [4].

Mulberry leaves also contain oxyresveratrol that play role as anti-inflammatory agents and also play role to inhibit cell T leukocytes migration through chemotaxis inhibition [9]. The discovery of antioxidant and anti-bacterial substances in the mulberry leaves extract (*Morus alba* l.) support it to prevent infectious disease including skin problem and wound healing process. If inflammatory process runs normally, the stimulation of various growth factors is not inhibited so it can process to the next phase (proliferation phase) quickly. Inflammatory phase can normally runs if there is no infection or damage that caused by free.

Proliferation phase is a repairment process that involves connective tissue with four components, namely new blood vessels formation, migration, and proliferation of fibroblasts, deposition of ECM (extracellular matrix) and maturation of fibrous tissue. Migration and proliferation of fibroblasts as well as ECM deposition of fibroblasts are two of the four components that play important roles in the proliferation phase [11]

Fibroblasts play an active role in the proliferation phase which started on the 3rd days along with the waning inflammatory phase and continues until the 14th day. The quantity of fibroblast in 20% mulberry ointment was higher than 10% mulberry ointment. Meanwhile, the percentage of collagen in 10% mulberry ointment was higher than 20% mulberry ointment. It was possibly caused by beyond the fibroblasts factors that able to influence the formation of collagen. The rest of it is influenced by other factors such as cytokines (IL-1, IL-4, IL-6, and Il-8) and growth factor (PDGF, FGF, IGf, and TGF-β) that able to influence the formation of collagen. However, this research did not do it due to limited funds. Factors that influence wound healing process are systemic factors such as age factor, nutrition factor, medications, glucose in the blood, Fe and Zinc micronutrient in the blood and also local factor that consist of blood supply, infection, necrosis, and unfamiliar object in the wound [10]

Limitation in this study was this research only studied the effect of ointment in 6 days and it did not study the effect of mulberry ointment to the peak and maturation proliferation phase.

CONCLUSION

There is an effect of 10% and 20% mulberry leaves extract ointment to the quantity of fibroblasts and collagen area fraction in the Sprague Dawley rat in the second-degree burn wound healing

SUGGESTION

Cytokine (Il-1, Il 4) and growth factor (FGF, PDGF, and TGF β) tests are required to determine the mechanism of collagen enhancement.

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The researcher has no conflict of interest in this publication

Authors' contribution

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Literature Review:
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Writing and Submitting manuscript:
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References:

1. Miller AC, Rashid RM, Mlis LF, Elamin EM, Zehtabchi S. Silver sulfadiazine for the treatment of partial-thickness burns and venous stasis ulcers. J Am Dermatology [Internet]. 2012;66(5):e159–65. Available from: http://dx.doi.org/10.1016/j.jaad.2010.06.014
2. Bhatia N, Singh A, Sharma R, Singh A, Soni V, Singh G, et al. Evaluation of burn wound healing potential of aqueous extract of Morusalba based cream in rats [Internet]. Vol. 3, The Journal of Phytopharmacology. 2014 [cited 2020 Apr 25]. Available from: www.phytopharmajournal.com
3. Nayak BS, Pinto Pereira LM. Catharanthus roseus flower extract has wound-healing activity in Sprague Dawley rats. BMC Complement Altern Med. 2006;6.
4. Thakur R, Jain N, Pathak R, Sandhu SS. Review Article Practices in Wound Healing Studies of Plants. Artic ID. 2011;2011:17.
5. Bhatia N, Sharma R, Kaur G, Kaur N, Singh G, Dhawan R. Beneficial effects of Morus alba and Azadirachtaindica leaf extract based combination cream in scalding type burn injury in rats. Int J Basic Clin Pharmacol. 2015;720–8.
6. Iwan J, Atik N. The Comparation Between Topical Aplication of the Aqueous Leaf Extract of Carica Papaya (ALEC) & Khaula Honey in Accelerating Skin Wound Healing in Mice. Maj Kedokt Bandung. 2010;42(2):76–81.
7. Goud PB,Kachole MS. Antioxidant enzyme changes in neem, pigeonpea and mulberry leaves in two stages of maturity. Plant Signal Behav. 2012;7(10):1258.
8. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Vol. 4, International Journal of Biomedical Science. Master Publishing Group; 2008. p. 89–96.
9. Chen YC, Tien YJ, Chen CH, Beltran FN, Amor EC, Wang RJ, et al. Morus alba and active compound oxyresveratrol exert anti-inflammatory activity via inhibition of leukocyte migration involving MEK/ERK signaling. BMC Complement Altern Med [Internet]. 2013 Feb 23 [cited 2020 Apr 25];13:45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23433072
10. Nagori BP, Solanki R. Role of medicinal plants in wound healing. Res J Med Plant. 2011;5(4):392–405.
11. Gonzalez ACDO, Andrade ZDA, Costa TF, Medrado ARAP. Wound healing - A literature review. Vol. 91, Anais Brasileiros de Dermatologia. SociedadeBrasileira de Dermatologia; 2016. p. 614–20.