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Original Research Article

Effect of Andong (*Cordyline fruticosa*) Leaf Extract on the Acceleration of Incised Wound Healing of Oral Mucosa in Wistar Rats (*Rattus norvegicus*)

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ABSTRACT

A wound is a condition of loss of cellular and anatomical or functional tissue. Fibroblasts are cells that dominate in the wound healing process, and they support capillary growth, collagen formation, and the formation of granulation tissue. Andong (*Cordyline fruticosa*) leaves are one of the plants used in herbal medicine. Their active substance content can stimulate the growth of new cells in wounds. This study was aimed at analyzing the histopathological effect of Andong leaf extract on the number of fibroblasts on incised gingival in Wistar rats (*Rattus norvegicus*). Thirty male Wistar rats were divided into six groups; three treatment and three control groups (5 rats in each group). The extract was prepared from the leaves of Andong. The wound was made on the labial gingival of the Wistar rats. The leaf extract was applied topically to the treatment group twice a day for 7, 14, and 21 days, while the control group was not given any treatment. Histological examination was performed on the wound tissue at the end of the experiment. The results indicated that there was an increase in the average number of fibroblasts on day 14th, with a further increase on day 21st in the control group. Meanwhile, the average number of fibroblasts was observed to increase on day 7, followed by a decrease on day 14, and finally, a further decrease on day 21 in the treatment group. The finding of this study showed that Andong leaf extract accelerates wound healing on the gingival of Wistar rats.

Keywords: Andong leaf extract, *Cordyline fruticosa*, Fibroblasts, Wound healing.

Introduction

Injury is a disruption of cell continuity that causes normal cells to be damaged. When the tissue is damaged due to injury, a wound healing process will occur. Wound healing is a complex relationship between cellular and biochemical actions that will begin the process of restoring the integration and functional security by regaining strength in protected tissue.[1] Wound healing process consists of four interrelated phases: hemostasis, inflammation, proliferation, and remodeling. In the proliferation phase, fibroblasts play an important role. Fibroblasts are cells that are widely distributed in tissues that produce collagen base fibers that link the wound edges. Also, they create new connective tissue and provide good protection to the wound to produce a good safety process. Increasing the number of fibroblast cells will increase the number of collagen fibers, thereby increasing the process of wound healing.[2,3] In producing an effective wound recovery, the body must supply materials and nutrients to the damaged area. Medicinal plants usually contain several ingredients and nutrients that are needed to help improve the treatment process. About 70% of proven drugs from plants are effective for healing wounds.[1,4] One of the plants that are widely used as traditional medicine is the Andong plant (*Cordyline fruticosa*). Traditionally, the Andong plant is used to treat pulmonary tuberculosis with cough blood, out spotting during pregnancy, bloody urine (haematuria), bleeding hemorrhoids, bleeding wounds, diarrhea, dysentery, and stomach pain.[5] The reason why attention needs to be paid to the healing of oral mucosal wounds is to accelerate healing with minimal scar formation, rapid recovery, prevention of invasion of colonies of microorganisms or other agents, into the tissue to prevent chronic inflammation. Andong plant extracts contain chemicals such as flavonoids, steroidal saponins, farrerol, quercetin helichrysoside, apigenin 8-C-*β*-D-glucopyranoside, and isoquercetin. The most widely used part of the Andong plant as a medicine is the leaf. The healing action of *C. fruticosa* is attributed to several properties. Fibroblasts are cells that dominate in the healing process. They support capillary growth, collagen formation, and granulation tissue formation. Saponins in Andong leaves have antibacterial activity. Also, they have an activity to increase fibroblast collagen synthesis, epithelial cell migration, and faster re-epithelialization.[5-12] Flavonoids reduce fibrosis in wound healing and correlate the effects of quercetin with changes in integrin expression on the fibroblast cell surface. Quercetin, a naturally occurring antifibrotic agent, reduces scar formation. It also increases surface integrin V and decreases integrin 1 in fibroblast cells. Changes in surface integrin expression may be a contributing factor to fibrosis including cell migration, proliferation, and extracellular matrix production. Quercetin can alter cell interactions with the extracellular matrix by regulating integrin expression to promote fibrosis reduction.[5-12]

The aim of this study was to analyze the histopathological effect of Andong (*Cordyline fruticosa*) leaf extract on the fibroblasts of incised gingival in Wistar rats (*Rattus norvegicus*).

Materials and Methods

Source of plant material

Andong leaves were collected from Lampinenung Banda Aceh in February 2014 and identified by Dr. Saida Rasnovi MSi of the Department of Biology, Faculty of Mathematics and Natural Sciences,

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with identification voucher number 6090/UN11.1.17/LT2013. The leaves were washed with water, separated from other unwanted plant parts, and dried for 1 week. Then, they were finely chopped and ground to a fine powder using a suitable grinder. The leaf powder was then stored in an airtight container and placed in a cool, dry, and dark place.

Source of experimental animal
Thirty male Wistar rats (*Rattus norvegicus*) aged 2-3 months, with a bodyweight of 150-250 g were used in this study. They were obtained from the Faculty of Veterinary, Syiah Kuala University. The rats were maintained at room temperature and fed *ad libitum.*

Ethical clearance
Ethical clearance for this study was obtained from the Faculty of Medicine Universitas Syiah Kuala with the ethical clearance number 217/KE/FK/2014.

Andong leaf extraction
Andong leaf extract was prepared by the maceration method. The powdered plant material (200 g) was weighed and macerated once in 2 L of 96% ethanol. The preparation was filtered, and the liquid extract was further concentrated using a vacuum rotary evaporator at 40°C to produce 100% Andong leaf extract.

Experimental grouping and treatment of Wistar rats
In this study, thirty Wistar rats were randomly divided into six treatment groups; I, II, III, and control groups IV, V, VI (5 Wistar rats/ group). The treatment group consisted of five rats each that were treated for 7, 14, and 21 days consecutively. Also, there were five rats each not treated (controls) for each of the treatment days. At the end of the experiment, the Wistar rats in each experimental group were anesthetized with an intramuscular injection of mixed xylazine (1-2 mg/kg), and ketamine (8-10 mg/kg). Then, the two groups were incised in the mandibular gingiva until it reached the 5 mm long alveolar bone. In the control group, after the labial gingiva was incised, the blood was cleaned and the extract was applied for 7, 14, and 21 consecutive days. In the treatment groups, after the labial gingiva was incised, the Andong leaf extract was applied to the wound 2 times daily (at 6 am and 6 pm) for 7, 14, and 21 days consecutively.

Histological examination
After the 7th, 14th, and 21st day, the rats were euthanized with 5% inhalation ether. Hematoxylin-eosin (HE) staining was carried out in 3 sequential stages of staining, dehydration, and purification. Staining was achieved with the main staining of hematoxylin for 10-15 min, then rinsed with water and alcohol alternately. Dehydration was carried out using alcohol in order of 70, 80, 96%, and finally, absolute alcohol for three min in each. Purification with xylol solution was performed for 60 min and repeated to get good and clear staining results. Histological observations in each group were made at 400x magnification to see the number of fibroblasts. The number of fibroblasts was observed on the slides using an Olympus CX 31 microscope with 400x magnification. Photographs were taken with an Olympus BX 41 microscope which was equipped with a DP-12 digital camera and OLYSIA software.[8]

Statistical analysis
Data analysis was performed using SPSS 17 and hypothesis testing was achieved with an unpaired t-test ($p < 0.05$).

Results and Discussion

The wound healing process is a complex cellular procedure that focuses on restoring the integrity of the forms and functions of injured tissue. The wound healing process begins immediately after the injury, but the repair mechanism of the damaged tissue depends on the type of wound. All tissues follow essentially the same pattern to complete the healing process with minimal scar formation. The oral cavity is a remarkable environment in which wound healing occurs in warm oral fluid, containing millions of microorganisms. Numerous studies on the potential of natural products with anti-inflammatory, antioxidant, antibacterial, and pro-collagen synthesis properties as wound healing agents have been undertaken.[13,14] The proliferative phase generally follows and overlaps with the inflammatory phase and is characterized by epithelial proliferation and migration over the provisional matrix within the wound (re-epithelialization). In the reparative dermis, fibroblasts and endothelial cells are the most prominent cell types present and support capillary growth, collagen formation, and the formation of granulation tissue at the site of injury.[2]
The results of the average value of the number of fibroblasts in Wistar rats on day 7 are presented in Table 1. There was a significant difference (p-value= 0.003) in the average number of fibroblasts observed in the treatment group (40 ± 5) compared to the control group (25 ± 5). Based on the histopathological observations (Figure 1A), it appeared that the number of fibroblasts in the treatment group was more than the ones observed in the control group. Clinically, the wound area did not appear to be completely closed in the control group, whereas in the treatment group, the wound appeared completely closed. On day 7, the process of wound healing was the proliferative stage. The proliferative stage aims to diminish the lesioned tissue area by contraction and fibroplasia, establishing a viable epithelial barrier to activate keratinocytes. This stage is responsible for the closure of the lesion itself, which includes angiogenesis, fibroplasia, and re-epithelialization. These processes begin in the microenvironment of the lesion within the first 48 hours and can unfold up to the 14th day after the onset of the lesion.[14]
On the 14th day after treatment, there was a significant difference (p = 0.028) in the mean number of fibroblasts observed in the control group which was 25.8 ± 4, while a value of 37.3 ± 6 was observed in the treatment group. The granulation tissue is formed through the following mechanisms: an increase in fibroblastic proliferation; collagenous and elastic biosynthesis, which creates a three-dimensional extracellular network of connective tissue; and the production of chemotactic factors and IFN-beta by fibroblasts. Endothelial cells and fibroblasts express integrin receptors and, through these, invade the coagulation found in the lesion area.[14]
The results of this study on the 21st day showed the average number of fibroblasts observed in the treatment group (21 ± 4.8) and the control group (32 ± 6.8) (p = 0.017) as displayed in Figure 2. The observations indicated that the number of fibroblasts in the treatment group was less than in the control group. This observation is because, on the 21st day after treatment, the wound enters the remodeling phase, marked by an increase in the amount of collagen and woven fiber to form new strength in scar tissue.

Table 1: Average number of total fibroblasts on day 7 of treatment

Variable	Group	Mean ± SD
Number of Fibroblasts	Treatment	40 ± 5
	Control	25 ± 5

P value = 0.003

Table 2: Average number of total fibroblasts on day 14 of treatment

Variable	Group	Mean ± SD
Number of Fibroblasts	Treatment	37.3 ± 6
	Control	25.8 ± 4

P value = 0.028

Table 3: Average number of total fibroblasts on day 21 of treatment

Variable	Group	Mean ± SD
Number of Fibroblasts	Treatment	21 ± 4
	Control	32 ± 6

P value = 0.017

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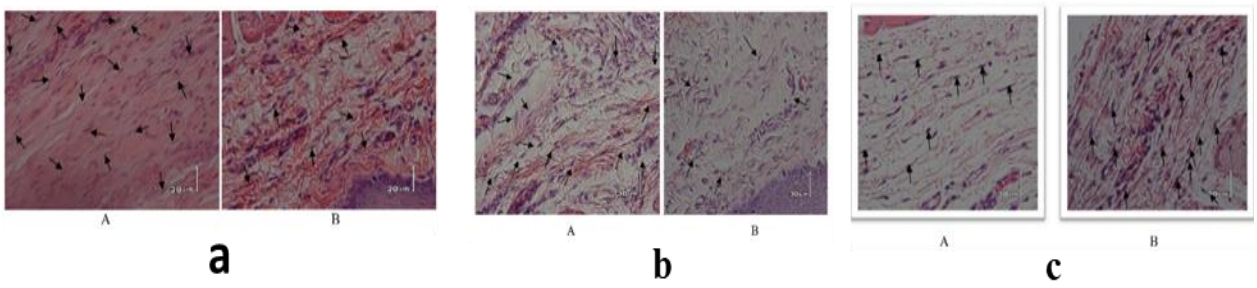

Figure 1: Histological comparison between increase in the number of fibroblasts in the Andong leaf extract treatment and control groups

a) On the 7th day: (A) Treatment group; (B) Control group. The number of fibroblasts was more in the treatment group compared with the control group.
b) On the 14th day: (A) Treatment group; (B) Control group. The number of fibroblasts in the treatment group was more than in the control group.
c) On the 21st day: (A) Treatment group; (B) Control group. The number of fibroblasts in the treatment group was less than in the control group.

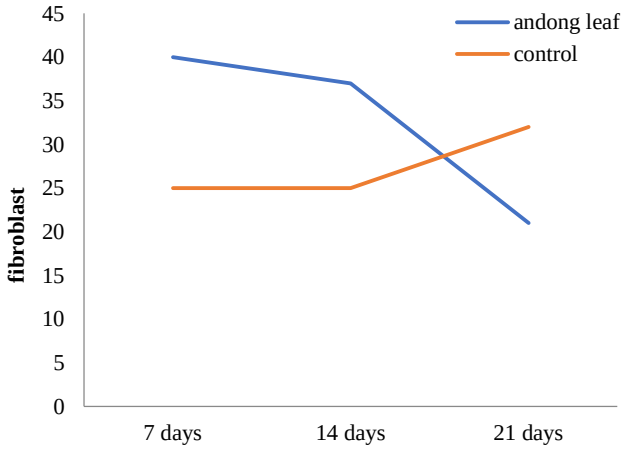

Figure 2: Comparison between increase in the number of fibroblasts in the Andong leaf extract treatment and control groups.

In the Andong leaf extract treatment group, there was an increase in the number of fibroblasts on the 7th day, which was followed by a decrease on the 14th day, and a further decrease on the 21st day. Meanwhile, in the control group, the number of fibroblasts increased on the 14th day, with a further increase on the 21st day but not as much as the number of fibroblasts obtained in the treatment group.

Besides, there was a decrease in the number of fibroblasts, macrophages, and angiogenesis as highlighted in Figure 2. The graph revealed that there was a decrease in the number of fibroblasts in the treatment group compared to the control group where it continued to increase. This signifies the third phase of wound healing. The third phase of healing consists of remodeling, which begins two to three weeks after the onset of the lesion and can last for one year or more. The main aim of the remodeling stage is to achieve maximum tensile strength through reorganization, degradation, and re-synthesis of the extracellular matrix. In the final stage of the lesion's healing, an attempt to recover the normal tissue structure occurs, and the granulation tissue is gradually remodeled, forming a scar tissue that is less cellular, vascular,[3] and that exhibits a progressive increase in its concentration of collagen fibers.[14]

After migrating to the injured area during the proliferation phase, fibroblasts begin the synthesis of the extracellular matrix and begin to enter the remodeling phase. Collagen is a protein from the extracellular matrix and is needed to provide strength when forming new connective tissue in the wound. In this phase, there is a regression of new blood vessels, so that the density of blood vessels in the wound returns to normal. The clinical feature of this phase is the formation of tissue that is close to normal by the extracellular matrix. The wound also experiences physical contractions throughout the wound healing process, which is believed to be mediated by fibroblasts observed in the wound. In incision wounds, there was almost no clinical difference between the treatment and control groups on the 21st day. In both groups, visible injuries that have been completely closed and scar tissue formed along the incision wound. However, histologically, the formation of connective tissue formation was almost completed in the treatment group, which was observed with the formation of a dense and regular woven collagen fiber. The results of this study showed differences in the appearance of fibroblasts in the control group which indicated collagen fibers that were not dense and irregular with fibroblast cells that were still widely distributed along the wound area. The 'holy grail' of wound healing is 'scarless wound healing': wound repair via the regeneration of functional, native tissue. Scarring and pathological wound healing states, such as hypertrophic scarring and keloids, represent an enormous clinical and financial burden on the healthcare system. Unfortunately, there are only a few effective therapies for hastening healing, while decreasing scarring.[15] The decrease in the number of fibroblasts in this study, in the treatment group showed that Andong leaf extract has the potential to minimize scarring. Quercetin is a component in the Andong leaf and a natural antifibrotic agent, which acts as a reduction in scar formation. Also, quercetin increases surface αV integrins and decreases β1 integrins on the surface of fibroblast cells. Changes in the surface integrin expression can be factors affecting fibrosis including cell migration, proliferation, and extracellular matrix production. Quercetin can alter cell interactions with the extracellular matrix by regulating integrin expression to reduce fibrosis.[7]

The observations made in this study are in agreement with the findings of many researchers investigating on wound healing process. Elzayat *et al* (2018) suggest that topical administration of the herbal gel formulation from henna, pomegranate, and myrrhic extract significantly improved wound healing in rats. They assessed the effect of the herbal material on the accelerated collagen deposition, formation of other connective tissue constituents, and antibacterial activity.[16] Hakim *et al* (2020) reported that 75% concentration of papaya extract showed perfect epithelialization, fibroplasia, and wound contraction in the *Mus musculus*.[8] Choii *et al*. showed that herbal mixture ointment from *Alchemilla vulgaris* and *Mimosa tenuiflora* (Mimosa) affected the wound healing process faster in mice. Their studies revealed that a mixture of ointments from *A. vulgaris* and *M. tenuiflora* (Mimosa) promoted re-epithelialization, collagen synthesis, and especially the regeneration of skin appendages.[17] Hakim *et al* demonstrated that *Glacilaria verucosa* gel influenced the wound healing process from the hemostatic phase, more fibrillation with minimal inflammatory cells in Wistar rats.[12] Santos *et al* in their study used *Vitis labrusca* leaf extract in experimental animals to assess the healing properties of oral administration of *V. labrusca* leaf hydroalcoholic extract. Their results indicated that oral administration of *Vitis labrusca* extract improved wound healing in rodents, histologically.[18]

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Conclusion

The findings from this study revealed that Andong leaf extract increases the number of fibroblasts of incision wounds in male Wistar rats that were observed. Also, the leaf extract proves to be effective in accelerating the wound healing process and minimizes scarring. The limitation of this study was that it only investigated the effect of the Andong leaf extract on fibroblasts in the wound healing process and did not observe other cells that also play an important role in the wound healing process. Also, the present study did not pay attention to the deeper molecular elements of these cells.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Rajsekhar S. Unseen aspects of wound healing: An overview. Int J Pharma Biosci. 2011; 2(9):275-287
2. Guo S and DiPietro LA. Factors affecting wound healing. J Dent Res. 2010; 89(3):219-229.
3. Masir O, Manjas M, Putra EA, Salmiah A Effect of culture filtrate fibroblast (CFF) fluid on wound healing. Experimental Research on Rattus Norvegicus Wistar J Kesehatan Andalas. 2012; 1(3):112-117.
4. Yadav KCH, Kumar JR, Basha SI, Desmukh GR, Gujjula R, Gujjula R, dan Santhamma B. Wound healing activity of topical application of aloe vera gel in experimental animal models. Int J Pharm Bio Sci. 2012; 3(2):63-72.
5. Dahlia AA, Ahmad AR, Wahid M. Extraction of color pigment and determination of flavonoid content of Andong leaves (*Cordyline Fruticosa L.)* source Makassar City. J Biol Sci Opin. 2013; 1(4):294-296.
6. Fouedjou RT, Nguelefack-Mbuyo EP, Ponou BK, Nguelefack TB, Barboni L, Tapondjou LA. Antioxidant activities and chemical constituents of extracts from *Cordyline fruticosa* (L.) A. Chev. (Agavaceae) and *Eriobotrya japonica* (Thunb) Lindl, (Rosaceae). Pharmacologia. 2016; 7(2-3):103-113.
7. Doersch KM and Newell-Rogers MK. The impact of quercetin on wound healing relates to changes in αV and β1 integrin expression. Exp Biol Med (Maywood). 2017; 242(14):1424-1431.
8. Hakim RF, Fakhrurrazi F, Dinni D. Effect of *Carica papaya* extract toward incised wound healing process in mice (*Mus musculus*) clinically and histologically. Evid-Based Compl Altern Med. 2019; 2019(5):1-5.
9. Fouedjou RT, Teponno RB, Quassinti L, Bramucci M, Petrelli D, Vitali LA, Fiorini D, Tapondjou LA, Barboni L. Steroidal saponins from the leaves of *Cordyline fruticosa* and their cytotoxic and antimicrobial activity. Phytochem Lett. 2014; (7):62-68.
10. Kim YS, Cho IH, Jeong MJ, Jeong SJ, Nah SY, Cho YS, Kim SH, Go A, Kim SE, Kang SS, Moon CJ, Kim JC, Kim SH and Bae CS. Therapeutic effect of total ginseng saponin on skin wound healing. J Ginseng Res. 2011; 35(3):360-367.
11. Gonzalez ACDO, Costa TF, Andrade ZDA, Medrad ARAP. Wound healing: A literature review. Bras Dermatol. 2016; 91(5):614-20.
12. Hakim RF, Fakhrurrazi F, Rezeki S, Sari LM, Marfirah Z. Hemostatic and wound healing effects of *Gracilaria verrucosa* extract gel in albino rats. Trop J Nat Prod Res. 2020; 4(11):912-917.
13. Politis C, Schoenaers J, Jacobs R, Agbaje JO. Wound healing problems in the mouth. Front Physiol. 2016; 7(507):1-13.
14. Ibrahim NI, Wong SK, Mohamed IN, Mohamed N, Chin KY, Nirwana SI, Shuid AN. Wound healing properties of selected natural product. Int J Environ Res Pub Health 2018; 15(2360):1-23.
15. Park HEdJ, Foster DS, Longaker MT. Fibroblasts and wound healing: an update. Reg Med. 2018; 13(5):491-495.
16. Elzayat EM, Sayed H. Auda, Fars K. Alanazi, Mohamed H. Al-Agamy, Evaluation of wound healing activity of henna, pomegranate and myrrh herbal ointment blend. Saudi Pharm J. 2018; 26(5):733-738.
17. Choi J, Park YG, Yun MS, Seol JW. Effect of herbal mixture composed of *Alchemilla vulgaris* and Mimosa on wound healing process. Biomed. Pharmacother. 2018; 106:326-332.
18. Santos TS, Santos IDD, Pereira-Filho RN, Gomes SVF, Lima-Verde IB, Marques MN, Cardoso JC, Severino P, Souto EB, Albuquerque-Júnior RLCd. Histological evidence of wound healing improvement in rats treated with oral administration of hydroalcoholic extract of *Vitis labrusca*. Curr Issues Mol Biol. 2021; (43):335-352.

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