Sys Rev Pharm 2020;11(5):567-570
A multifaceted review journal in the field of pharmacy

The Effect of Topical Curcumin Extract on Fibroblast Count and Collagen Density as an Indicator on Accelerating Clean Wound Healing Process: A Study on Wistar Rats

Erick Berto Yahya, Ariandi Setiawan*, Marjono Dwi Wibowo, Edwin Danardono

Department of Surgery, Faculty of Medicine-Dr. Soetomo Teaching Hospital, Universitas Airlangga, Surabaya 60131, Indonesia

ABSTRACT

A factor that plays an important role in the process of wound healing is fibroblast, where fibroblasts can trigger the formation of collagen. Turmeric (*Curcuma longa*) containing curcumin and volatile oil is believed to accelerate fibroblast proliferation and collagen synthesis. This study compared the effectiveness of curcumin extract topical and tulle in the acceleration the process of wound healing in rat skin. This was an experimental study using *Rattus novergicus*. The rats were divided into four groups: group of wounds that treat with carbomer, NaCl 0.9%, tulle and curcumin extract topical, then they were compared by the fibroblast count and collagen density. In this study, there were significant differences in the number of fibroblasts among the four groups (p = 0.034). We found that mean fibroblast count in group of wounds treated with curcumin extract topical was higher than that treated with tulle, and we obtained p = 0.045. There was no difference in collagen density score between groups of wounds treated with curcumin extract topical and tulle. There was a significant difference of fibroblast count in curcumin extract topical group compared to tulle group. However, there was no significant difference in collagen density score between both of them.

Keywords: Fibroblast, collagen, curcumin extract topical, tulle, wound healing

Correspondence:
Ariandi Setiawan, MD
Department of Surgery, Faculty of Medicine-Dr. Soetomo Teaching Hospital, Universitas Airlangga, Surabaya 60131, Indonesia
Tel: +6231-550-1327; Fax: +6231-504-9256
E-mail: ariandi.setiawan@gmail.com

INTRODUCTION

Sometimes, wounds, such as punctures, sharp cuts, or surgical injuries, cannot be avoided (1). Wounds are the main cause of disability which requires a person to care of. The long duration of wound treatment will increase the cost of patient care in the hospital. The entire wound healing process involves a series of complex events that begin at the time of the injury and can continue for months to years (2). Wound healing can be influenced by many factors, both local and systemic (3–5). Recovery is one of the physiological processes after surgery. Injuries that are resolved successfully for a long time are often a problem. This incident will cause complications as a source of morbidity, because it will be psychological disturbances for sufferers, increase the budget for treatment costs, and reduce working hours for patients in productive age (6). Fibroblasts are cells that synthesize extracellular and collagen matrices, playing an important role in the process of wound healing. The number of fibroblasts reaches a peak on the 7th day after trauma and is the dominant cell in the first week of the wound healing phase [1]. Synthesis and activation of fibroblasts are performed by secretory proteins and macrophages of new extracellular matrix approvals with collagen as the structure of consent (7). Traditional wound dressing using only normal saline is relatively cheaper but has a longer shortage of wound dressing compared to modern wound dressing. In modern wound dressing, such as tulle, has synthetic polymer base material not only closes the wound but also spur the wound care (8–10).

Turmeric (*Curcuma longa*) is a rhizome plant widely known throughout the world. One of the active substances contained in turmeric is curcumin. Curcumin accelerates fibroblast proliferation and collagen synthesis and maturation (11,12). Curcumin also contains anti-allergy and anti-bacterial activity (13,14). Hence, this study aimed to examine the comparison between turmeric extract herbal products and modern synthetic dressing tulle against the number of fibroblasts and collagen density in accelerating the healing of clean wounds on rat skin experimentally [2].

METHODS

This was an experimental study by administering topical turmeric and tulle extracts to clean rat skin wounds. The design used was a randomized posttest-only control group design. The measurement of variables was only performed at the end of the study. The sample of the study was 36 white *Rattus novergicus* Wistar strain that met the inclusion and exclusion criteria. Criteria for inclusion of male Wistar strain white rats were age of 12-16 weeks and body weight of 250-300 grams. The exclusion criteria were during the adaptation period to behave and dressing on the wound released before the dressing replacement schedule for the next wound. The samples were divided into 4 groups based on the sample sequence number interval. The positive control was wound care using 0.9% NaCl fluid without the addition of tulle products or topical turmeric extract. Wound care was also used by adding a basic ingredient, carbomer, as a negative control variable to reduce bias. Treatment group 1 treated wound with the addition of tulle, while treatment group 2 treated wound with the addition of topical turmeric extract. The independent variable in this study was the administration of topical turmeric and tulle extracts. The dependent variables were the number of fibroblasts and collagen density assessed histopathologically. Assessment criteria was with a scoring system.

RESULTS

In this study, the number of fibroblasts in the area was measured by wound edges on the 6th day by comparing into 4 groups. Based on the characteristics and relationships of the number of fibroblasts between the 4 groups in Table 1, the mean number of fibroblasts of the negative control group was 68.44±6.09, the positive control group was 70.00±23.08, the treatment group 1 was 71.22±24.26, and the treatment group 2 was 88.56±9.08. The number of normal and homogeneous distributed fibroblasts were examined using ANOVA statistical test, and there were significant differences in the number of fibroblasts among four groups (p = 0.034).

Table 1. Number of fibroblasts

Groups	Mean±SD	Min-Max	p
Negative control (N=9)	68.44±6.09	58-78	0.034*
Positive control (N=9)	70.00±23.08	44-101	
Treatment 1 (N=9)	71.22±24.26	30-103	
Treatment 2 (N=9)	88.56±9.08	72-102	
*) Anova Test			

Table 2 presents the difference of the number of fibroblasts among four groups. The treatment group 2 had the highest number of fibroblasts and was statistically significant compared to the other groups (p = 0.021 compared to the negative control group, p = 0.033 compared to the positive control group, and p = 0.045 compared to treatment group 1).

Table 2. The difference of the number of fibroblasts

Groups		Mean Difference	p
Control Negative	Control Positive	-1.555	0.853
	Treatment 1	-2.777	0.740
	Treatment 2	-20.11	0.021
Control Positive	Negative control	1,555	0.853
	Treatment 1	-1.222	0.884
	Treatment 2	-18.555	0.033
Treatment 1	Negative control	2.777	0.740
	Treatment 1	1.222	0.884
	Treatment 2	-17.333	0.045
Treatment 2	Negative control	1,555	0.853
	Treatment 1	-1.222	0.884
	Treatment 2	-18.555	0.033
Confidence Interval = 95%			

In this study, an evaluation of collagen density was carried out on the 6th day by comparing into 4 groups as seen in Table 3. In the negative control group, it was found that the highest score was 3 in 5 samples (55.6%), followed by a score of 2 in 4 samples (44.4%), and no sample was found at a score of 1 (0%). Meanwhile, the positive control group found the highest score of 3 in 5 samples (55.6%), followed by a score of 2 in 4 samples (44.4%). In the treatment group 1, the highest score was 3 in 6 samples (66.7%), followed by score 2 in 3 samples (33.3%), and no sample was found in score 1 (0%). Meanwhile, the treatment group 2 was found the highest score of 3 in 6 samples (66.7%), followed by a score of 2 in 3 samples (33.3%), and no sample was found at a score of 1 (0%). In this study, the difference in collagen density test was examined using the Kruskal Wallis test. There was no significant difference in collagen density in the four groups (p = 0.929).

Table 3. Collagen Density Score

Groups	Score 1	Score 2	Score 3	p
Negative Control	0 (0%)	4 (44.4%)	5 (55.6%)	0.929*
Positive Control	0 (0%)	4 (44.4%)	5 (55.6%)	
Treatment 1	0 (0%)	3 (33.3%)	6 (66.7%)	
Treatment 2	0 (0%)	3 (33.3%)	6 (66.7%)	
*) Kruskal wallis test				

DISCUSSION

We found the number of fibroblasts of curcumin treatment increased more than in the other groups. There was a significant difference in the number of fibroblasts in the topical turmeric extract group compared to the other groups. The results of this study are in line with the recent study. The curcumin can increase the number of fibroblasts [3]. This is because curcumin plays a role in inhibiting the production of Tumor Necrosis Factor-alpha (TNF-α) and Interleukin-1 (IL-1) as the two main cytokines released by monocytes and macrophages, playing a role in inducing the inflammatory response. With the inhibition of production of proinflammatory cytokine, inflammatory cells, such as polymorphonuclear, will be reduced in the wound area, thus shortening the length of the inflammatory phase and accelerating the wound healing process to enter the next healing phase, namely the proliferation phase with the formation of fibroblasts (15). Another study also showed mice given curcumin topically in excision wounds made, showing that fibroblasts formation proceeded better than wounds treated only using ordinary gauze and primary suturing (16). Besides curcumin, there are also some plants with wound healing effect, such as aloe vera and mangosteen (*Garcinia mangostana* Linn.) (17,18).

The number of fibroblasts has a quite different deviation. This is presumably because some animals try to get interference from the behavior of other animals in a cage which can be caused by hot environmental conditions and drinking water and feed for each mouse in a cage that is not evenly distributed because one cage contains four and five mice. In treatment group 1, we applied tulle [4]. Tulle is a synthetic textile fiber which is a polyamide resin derivative with elastic characteristics, having anti-penetrating properties and containing lanolin. Lanolin will create a moist atmosphere by creating a semi-occlusive barrier, preventing the wound from drying out and accelerating re-epithelialization and proliferation of fibroblasts. While in treatment group 2, we used topical turmeric extracts. Turmeric extract has many active components, such as curcumin, desmethoxycurcumin and bisdemethoxycurcumin which function as antioxidant and anti-inflammatory effects. In addition to turmeric extract, there is another main active component, essential oil. Essential oil also has antioxidant and anti-inflammatory effects to improve the wound healing process (11,19–21).

Wound healing is the body's process to repair damaged tissue to function again. In normal condition, wound can heal by itself, but how fast it is can be affected by many factors (22). The body tries to normalize all abnormal conditions due to injury by the healing process. The body responds if the integrity of the skin is damaged in the form of overlapping phases, but can be distinguished biologically. After injury, the inflammatory phase occurs aiming to eliminate non-vital tissue and prevent invasive bacterial infections. Then, there is a proliferation phase where there is a balance between the formation of scar tissue and tissue regeneration. Finally,

there is a renovation phase which aims to maximize the strength and structural integrity of the wound. These phases overlap with each other, and begin when the injury occurs until the resolution of the wound (23,24). The proliferation phase is the most important phase in wound healing. The proliferation phase lasts from day 4 to day 21 after the injury. The important thing to consider in the proliferation phase is that when collagen has filled the wound completely, fibroblast activity will stop and this is a physiological process in the human body to prevent excessive wound healing. Fibroblasts are the main cell during this phase providing a framework for keratinocyte migration (7). Fibroblasts will produce the basic ingredients of collagen fibers that will link the wound edges. With the influence of growth factors and hydrolytic enzymes released by macrophages, fibroblasts proliferate and produce lots of collagen (25).

The study also showed that there was an increase in collagen density scores in wounds given curcumin and tulle compared to wounds in the control group, but there was no significant difference in collagen density in the four groups. Another study tested the effect of curcumin in turmeric on collagen formation. The study examined mice treated for skin injury and given topical curcumin therapy and found that there was collagen formation with collagen maturation occurring faster than mice that did not receive wound care with topical curcumin (16). The curcumin can accelerate the wound healing process by inducing more TGF-β1 formation a lot compared to wound care without using curcumin turmeric extract. Increasing levels of TGF-β1 and growth factors in the wound area increases the proliferation and migration of fibroblasts and induces ECM synthesis in the proliferation phase, so that collagen production increases (26-30). However, in this study, the results showed that collagen density was not significant in the statistical tests of the four groups. This could be due to the time of the initial study sampling, which is on the 6th day where collagen has not been formed optimally (7).

CONCLUSION

There was a significant difference in the number of fibroblasts in the topical turmeric extract group compared to the groups of carbomer administration, NaCl 0.9%, and tulle. There was no significant difference in collagen density scores in all treatment groups. However, tropical and tulle turmeric extract had higher collagen density scores compared to the control group.

REFERENCES

1. Irham WH, Tamrin, Marpaung L, Marpongahtun. Bioactive Compounds in Pegagan Leaf (Centella asiatica L. Urban) for Wound Healing. In: Journal of Physics: Conference Series. Institute of Physics Publishing; 2019.
2. Hanafiah OA, Abidin T, Ilyas S, Nainggolan M, Syamsudin E. Wound healing activity of binahong (Anredera cordifolia (Ten.) Steenis) leaves extract towards NIH-3T3 fibroblast cells. J Int Dent Med Res. 2019;12(3):854–8.
3. Guo S al, DiPietro LA. Factors affecting wound healing. J Dent Res. 2010;89(3):219–29.
4. Hajialyani M, Tewari D, Sobarzo-Sánchez E, Nabavi SM, Farzaei MH, Abdollahi M. Natural product-based nanomedicines for wound healing purposes: therapeutic targets and drug delivery systems. Int J Nanomedicine. 2018;13:5023.
5. Järbrink K, Ni G, Sönnergren H, Schmidtchen A, Pang C, Bajpai R, et al. The humanistic and economic burden of chronic wounds: a protocol for a systematic review. Syst Rev. 2017;6(1):15.
6. Shanmugam MK, Rane G, Kanchi MM, Arfuso F, Chinnathambi A, Zayed ME, et al. The multifaceted role of curcumin in cancer prevention and treatment. Molecules. 2015 Feb;20(2):2728–69.
7. Gurtner GC. Wound healing: normal and abnormal. Grabb Smith's Plast Surgery-Philadelphia, LWW. 2014;13–9.
8. Atiyeh BS, Al-Amm CA, Nasser AA. Improved healing of split thickness skin graft donor sites. J Appl Res. 2002;2(2):114–21.
9. Dhivya S, Padma VV, Santhini E. Wound dressings–a review. BioMedicine. 2015;5(4).
10. Weller C, Sussman G. Wound Dressings Update. J Pharm Pract Res [Internet]. 2006 Dec 1;36(4):318–24. Available from: https://doi.org/10.1002/j.2055-2335.2006.tb00640.x
11. Akbik D, Ghadiri M, Chrzanowski W, Rohanizadeh R. Curcumin as a wound healing agent. Life Sci. 2014;116(1):1–7.
12. Emiroglu G, Ozergin Coskun Z, Kalkan Y, Celebi Erdivanli O, Tumkaya L, Terzi S, et al. The effects of curcumin on wound healing in a rat model of nasal mucosal trauma. Evidence-Based Complement Altern Med. 2017;2017.
13. Usamah U, Juniastuti J, Ramadhani RB. Antibacterial Activity Of Kunyit (Curcuma Longa) Leaves Extract On Staphylococcus Aureus Examined Using Dilution Method. Folia Medica Indones. 2012;48(4):163.
14. Krismariono A. The decreasing of NFκB level in gingival junctional epithelium of rat exposed to Porphyromonas gingivalis with application of 1% curcumin on gingival sulcus. Dent J (Majalah Kedokt Gigi). 2015;48(1):35–8.
15. Kant V, Gopal A, Pathak NN, Kumar P, Tandan SK, Kumar D. Antioxidant and anti-inflammatory potential of curcumin accelerated the cutaneous wound healing in streptozotocin-induced diabetic rats. Int Immunopharmacol. 2014;20(2):322–30.
16. Mohanty C, Das M, Sahoo SK. Sustained Wound Healing Activity of Curcumin Loaded Oleic Acid Based Polymeric Bandage in a Rat Model. Mol Pharm [Internet]. 2012 Oct 1;9(10):2801–11. Available from: https://doi.org/10.1021/mp300075u
17. Machmud E, Jubhari EH, Ardiansyah M. Histometry analysis of osteoclasts, osteoblasts and fibroblasts in immediate placement implants with addition of aloe vera extract. Pesqui Bras Odontopediatria Clin Integr. 2019;19(1).
18. Sombolayuk HB, Djawad K, Wahab S, Miskad UA, Alam G, Pattelongi I. The effectivity of topical mangosteen pericarp extract cream on wound healing in Swiss albino mice. J Biol Res. 2019;92(2).
19. Kocaadam B, Şanlier N. Curcumin, an active component of turmeric (Curcuma longa), and its effects on health. Crit Rev Food Sci Nutr. 2017;57(13):2889–95.
20. Sood A, Granick MS, Tomaselli NL. Wound Dressings and Comparative Effectiveness Data. Vol. 3, Advances in wound care. 2014. p. 511–29.
21. Stanojević J, Stanojević L, Cvetkovic D, Danilovic B. Chemical composition, antioxidant and antimicrobial activity of the turmeric essential oil (Curcuma longa L.). Adv Technol. 2015 Jan 1;4:19–25.
22. Laila L, Febriyenti F, Salhimi SM, Baie S. Wound healing effect of Haruan (Channa striatus) spray. Int Wound J. 2011;8(5):484–91.

23. Gonzalez AC de O, Costa TF, Andrade Z de A, Medrado ARAP. Wound healing - A literature review. An Bras Dermatol. 2016;91(5):614–20.
24. Brunicardi F, Andersen D, Billiar T, Dunn D, Hunter J, Matthews J, et al. Schwartz's principles of surgery, 10e. McGraw-hill; 2014.
25. Mescher AL, Junqueira S. Basic histology text and atlas. Medical McGraw Hill eBook. Indiana; 2013.
26. Maheshwari RK, Singh AK, Gaddipati J, Srimal RC. Multiple biological activities of curcumin: a short review. Life Sci. 2006;78(18):2081–7.
27. Medina, C.M.A., et al., *REHABILITATION OF EXECUTIVE FUNCTIONS AND SOCIAL COGNITION, IN PEOPLE WITH ANTISOCIAL PERSONALITY DISORDER, LINKED TO THE ARMED CONFLICT IN COLOMBIA/REHABILITACIÓN DE LAS FUNCIONES EJECUTIVAS Y LA COGNICIÓN SOCIAL, EN SUJETOS CON TRASTORNO DE PERSONALIDAD ANTISOCIAL, VINCULADAS AL CONFLICTO ARMADO EN COLOMBIA.* Revista Argentina de Clínica Psicológica, 2019. **28**(I): p. 92.
28. Duarte, S., G. Martinez, and S. Tomicic, *RELEVANT EPISODES AND EVENTS FOR THE STUDY OF CHANGE IN PSYCHOTHERAPY.* REVISTA ARGENTINA DE CLINICA PSICOLOGICA, 2019. **28**(3): p. 253-265.
29. Bautista Menares, D. and L. Contreras Taibo, *PSYCHOSOCIAL EFFECTS ON CHILDREN AND ADOLESCENTS INDIRECT VICTIMS OF INTIMATE HOMICIDE OF THEIR MOTHER.* REVISTA ARGENTINA DE CLINICA PSICOLOGICA, 2019. **28**(3): p. 266-274.
30. Cebrian, P.R., et al., *PSYCHOLOGICAL GROUP INTERVENTION IN PEOPLE WITH PSORIASIS: EFFECTIVENESS OF A PILOT PROGRAM.* REVISTA ARGENTINA DE CLINICA PSICOLOGICA, 2019. **28**(3): p. 275-284.