

Efficacy of Oregano Extract Ointment on Fibroblast Cells and Epidermis in Albino Rats with Excisional Wound Model

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Abstract

This study aimed to determine the effect of oregano extract ointment therapy on the number of fibroblast cells and the epidermal thickness in excised wounds. The experimental animals used were 20 male Wistar strain albino rats, 12 weeks old, weighing 100–150 g, divided into 5 treatment groups i.e., (C-) not excised and without ointment, (C+) excised and without ointment, and the treatment group were excised using a 5 x 5 mm biopsy punch and treated with oregano extract ointment using concentrations of (T1) 3%, (T2) 6% and (T3) 9% twice daily for 14 days in an excised wound. The variables observed in this study were the number of fibroblast cells and the epidermal thickness by the HE staining method then measured using ImageJ. Data analysis used the One-Way ANOVA test followed by the Tukey test ($p < 0,05$). The results showed a decrease in the number of fibroblast cells and an increase in the epidermal thickness in the 6% concentration ointment. This study concluded that the administration of oregano extract ointment with a concentration of 6% was the optimal concentration in accelerating the final proliferative phase, characterized by a decrease in fibroblast cells and an increase in epidermal thickness.

Keywords: epidermal thickness, fibroblast, oregano ointment, *Origanum vulgare*

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INTRODUCTION

The physiological and anatomical functioning of bodily components can be disturbed by wounds, which are physical traumas (Mickelson *et al.*, 2016). Injuries come from tissue disruption brought on by mechanical trauma, explosions, chemical exposure, temperature fluctuations, wounds, or bites (Fazri, 2019). A wound that removes tissue from the epidermal layer to the subcutaneous layer is an example of an excision wound (Andriani, 2019). In reaction to a wound, the body will go through a biological process called wound healing. This process will subsequently repair the anatomy of the skin's cell structures and tissue layers as well as its physiology (Samirana *et al.*, 2016). The process of healing a wound can be aided chemically, such as with medications, ointments, and the like, or naturally (Qomariah, 2014). According to Naibaho *et al.* (2013), ointment is a topical treatment comprised of therapeutic components dissolved in an ointment base that

contains the active ingredient. Due to the fact that there is no first-pass removal and that topical treatment with ointments has a local effect, it might hasten medication absorption into the skin and be widely absorbed into the circulation (Octasari and Ayuningtyas, 2016).

Hemostasis, inflammation, proliferation, and remodeling are stages of the wound healing process. Coagulation that follows tissue injury leads to the development of a fibrin clot. Damaged tissue or pathogen detection triggers the release of local cytokines and growth factors, which starts the inflammatory phase of wound healing and attracts circulating innate immune cells. To stop further tissue damage from causing persistent, chronic inflammation to proceed from acute inflammation to chronic inflammation, the inflammatory response must be inhibited (Chen *et al.*, 2018). The presence of anti-inflammatory flavonoids during wounds allows them to lower inflammatory mediators and hasten the healing process so that it may quickly move through the

inflammatory phase and into the proliferative phase (Al Ghifari, 2021).

The proliferation phase, which comes after the inflammatory phase is through, is characterized by fibroblast activity, which secretes components of the extracellular matrix (ECM), the building blocks of newly formed tissue (Kartikasari *et al.*, 2020). Blood vessels and loose connective tissue fibers called granulation tissue will start to fill the wound and form a structure that will eventually serve as a pathway for fibroblasts and epithelial cells to move through (Dewi *et al.*, 2023). The differentiation of fibroblasts into myofibroblasts, which store structural macromolecules such as collagen fibers and glycosaminoglycans, is promoted by a variety of cytokines and growth factors (Purnama *et al.*, 2019; Steen *et al.*, 2020).

In Indonesia, the usage of herbal remedies as complementary therapies is still expanding (Hamiyati and Laratmase, 2021). The Lamiaceae family of medicinal plants, which is well recognized for being a plant that is frequently used to boost spicy taste and aroma, includes oregano (*Origanum vulgare*) as one of their members. The oregano plant contains a number of bioactive substances that have strong antibacterial, antifungal, and anti-inflammatory activity, including phenolic acids, phenolics, esters, flavonoids, and steroids (Sankar *et al.*, 2014). Carvacrol and thymol, two antibacterial compounds found in oregano extract have anti-inflammatory properties (Lordani *et al.*, 2018).

Oregano extract contains flavonoid components that can boost macrophage metabolism and encourage fibroblast cell activation to generate granulation tissue (Prestiyanti, 2021). Alkaloid substances will hasten the re-epithelialization phase, increasing the production of epithelial cells in the epidermis and raising the epidermal thickness. Because saponin can boost the expression of B-cell lymphoma 2 in keratinocytes and produce TGF-1 and VEGF, which function as growth factors and are responsible for the re-epithelialization phase, saponin can have a role in promoting epidermal cell proliferation. Tannins can promote the growth of epithelial cells during the healing phase

of a wound, aid fibroblast migration into the wound, and promote collagen synthesis during the proliferation phase (Al Ghifari, 2021). Based on this, a study was done to ascertain the impact of oregano extract ointment on the quantity of fibroblast cells and skin epidermal thickness in rats using an excision wound model.

MATERIALS AND METHODS

Experimental Animals

The test animals in this research were male Wistar rats, aged 12 weeks with a body weight of 100–150 grams and were in good health. In the initial stage, rats were adapted for 7 days, and during the maintenance period, they were given basal food and drink ad libitum (Adiguna, 2019). The experimental animals consisted of 5 treatment groups; (C-) not excised and without ointment, (C+) excised and without ointment, and the treatment group was excised using a 5 x 5 mm biopsy punch and treated with oregano extract ointment using concentrations of (T1) 3%, (T2) 6% and (T3) 9% twice daily for 14 days in an excised wound (Upa *et al.*, 2017).

Excisional Wounds in Experimental Animals

The experimental animal treatment was approved by the Brawijaya Research Ethics Commission No.126-KEP-UB-2022. Rats were anesthetized using Ketamine and Xylazine at a dose of 80 mg/kg BW and 8 mg/kg BW. Clean the dorsal area and shave the hair to an area of around 3 cm x 3 cm. After that, disinfection was carried out using 70% ethanol. Excision wounds were carried out using a 5 mm punch biopsy on the skin of the left and right bilateral back areas. This excision is carried out by taking skin tissue by pressing the punch biopsy on the epidermis to the hypodermis of the subcutaneous layer (Sartika *et al.*, 2020).

Oregano Extract Production

A total of 500 g of dry oregano leaves were weighed and then put in a closed container to make oregano extract. The dried oregano leaves were then macerated in 1000 cc of 96% ethanol until completely submerged, and the mixture was

then allowed for 24 hours. Furthermore, the mixture was shaken at a speed of 50 rpm in a digital shaker and then filtered, and the cloth specimens were stored in an Erlenmeyer tube. After that, rotational evaporation was performed using low pressure at a temperature of 70°C (Mufid, 2018).

Production of Oregano Extract Ointment

A total of 10 g of oregano extract ointment was carried out by weighing the vaseline album, then put into a porcelain cup to be melted in a water bath. After the ointment base has melted, it can be homogenized by stirring in a mortar. Next, oregano extract can be added according to the concentration and then stirred until homogeneous (Zulfa *et al.*, 2015).

Oregano Extract Ointment Therapy

Oregano extract ointment therapy was given to excision wounds in graded concentrations, i.e., (T1) 3%, (T2) 6%, and (T3) 9%. The ointment was applied topically twice a day using a cotton bud on the excision wound area for 14 days after skin excision.

Rat Skin Preparations

Rats were euthanized using the cervical dislocation method. Next, the rat was positioned lying ventrally on a fixation board, and then the hair on the rat's back was cleaned and shaved. Skin tissue was removed by cutting at a distance of 1 cm around the excision wound. Then the tissue was fixed using 10% formalin for 48 hours.

Histopathology Preparations

Skin tissue was trimmed on the skin tissue. Then the dehydration process was carried out by soaking in ethanol solution with graded concentrations, i.e., 70%, 80%, 85%, 90%, 95%, and 100% ethanol (I, II, and III) for 1 hour. After the dehydration process was completed, then soaked in xylol I, xylol II, and xylol III liquid for 15 minutes (Westri, 2018). The paraffinization process was performed using liquid paraffin I, II, and III for 30 minutes respectively. Furthermore, the embedding process was carried out in paraffin until it solidified and the tissue was cut in 5 µm

thick paraffin using a microtome and attached to a glass object. The tissue was heated on a glass object at a temperature of 56–58°C until the remaining paraffin melted (Westri, 2018). Next, the deparaffinization process was carried out by immersing the slides in xylol I, II, and III and continued with the rehydration process using ethanol in descending concentrations, i.e., 100% absolute ethanol (I, II, and III), 95%, 90%, 80%, and 70%. HE staining was stained the slide in Hematoxylin solution for 15 minutes, soaked in acidic alcohol for 4 seconds, then washed using water for 20 minutes and soaked in Eosin solution for 15 minutes. Dehydration was carried out with ethanol in graded concentrations of 70%, 80%, 90%, and 95% and absolute alcohol I, II, and III, for four seconds each. The clearing was carried out with xylol I, II, and III solutions for 20 minutes and continued with the mounting stage using Entelan and covered with a cover glass (Kusumawardhani *et al.*, 2015).

Evaluation of Fibroblast Cells

Fibroblast cells were observed using a Nikon Trinocular microscope with a Nikon DS-Fi3 digital camera with a total magnification of 400x with five fields of view in the excision wound area. After obtaining histopathological images, fibroblast cells were counted using ImageJ software.

Evaluation of Epidermal Thickness

Observations of epidermal thickness were observed using a Nikon Trinocular microscope with a Nikon DS-Fi3 digital camera with 400x magnification in five fields of view, each of which was measured at three different points and then the average was taken. The epidermal thickness was measured from the stratum corneum to the stratum basale using the ImageJ application.

Data Analysis

Data analysis used the One-Way ANOVA test ($\alpha = 5\%$) followed with the Tukey test $p < 0,05$ using Statistical Package for Social Science (SPSS) software to determine significant differences in the treatment groups (Mihmidati and Athiroh, 2017).

RESULTS AND DISCUSSION

Fibroblasts are spindle-shaped cells located in the dermis layer. These cells are of mesenchymal origin which play a role in the degradation of fibrin clots, the production of a large number of cytokines and growth factors, and the formation of granulation tissue. In injured tissue, fibroblasts will be activated and differentiate into myofibroblasts. Myofibroblasts will contract, reducing the size of the wound and releasing ECM proteins (Bin and James, 2011). Collagen produced from fibroblast cells which is the most common form of extracellular sheath and forms fibers in the extracellular environment (Nilforoushzadeh *et al.*, 2017).

Tukey test results showed that group C- was significantly different from groups C+, T1, and T3. The T2 group was significantly different from the C+ group. The average number of fibroblast cells in the T2 group was $62,25 \pm 11,558$, which was the closest increase to the average value of the C- group (Table 1 and Figure 1). This study showed that the T2 group experienced a faster final proliferation phase so the administration of Oregano extract ointment with a 6% concentration was the most effective result in the wound healing process. The final stage of proliferation towards maturation is marked by a decrease in the number of fibroblast cells which regenerate into fibrocyte cells, then the fibroblasts will secrete collagen and form granulation tissue until the wound surface closes (Prestiyanti, 2021; Suryadiningrat *et al.*, 2021). Tannins inhibit the attachment of bacteria to the surface of skin wounds. Inadequate bacterial adhesion to the skin surface causes bacterial cell death (Kaczmarek, 2020). Tannins play a role in stabilizing the formation of collagen and elastin in the extracellular matrix. This is demonstrated by inhibiting Matrix Metalloproteinase (MMP-9) collagenase which is accompanied by increased collagen binding (Orlowski *et al.*, 2018). Other active ingredients in Oregano extract which are also extracted with 96% ethanol are carvacrol and tannin. Carvacrol can inhibit the expression of IL-4, COX-2, and TNF- α . Thymol is able to reduce

the expression of TNF- α , IL-6, IL-1 β , and reduce inflammation (Nilforoushzadeh *et al.*, 2017; Costa *et al.*, 2019).

The thickness of the epidermis will indicate a re-epithelialization process which is a stage in the wound healing process through mitosis, migration, and differentiation of epithelial cells in replacing lost and damaged skin integrity. The re-epithelialization process will re-form the skin wound layer with new epithelium from the edge of the wound towards the center to create epidermal regeneration (Sukmanadi *et al.*, 2021). In the re-epithelialization phase, matrix formation will occur, migration of epidermal keratinocytes from the wound edge, and keratinocyte proliferation (Rousselle *et al.*, 2019). Growth factors will play a role in stimulating the proliferation of epithelial cells, for example, epidermal growth factor, transforming growth factor, and keratinocyte growth factor (Triana *et al.*, 2020). Group C- is an indicator of normal epidermal thickness, which ranges from 10–15 μm (Rosellini, 2017).

Tukey test results showed that group C- was significantly different from groups C+, T1, T2, and T3 due to the rats having normal skin without any excision treatment. The T2 group showed a significant epidermal thickness of 54,99 μm . The T3 group was reported to have the lowest epidermal thickness among the C+, T1, and T2 groups (Table 1 and Figure 2). This can occur due to factors in the ointment formulation with a high extract concentration which will have a high ointment viscosity (Afianti and Murrkmiyadi, 2015). A high extract concentration causes the amount of water in the ointment base to decrease so that the ointment becomes thicker (Ulandari and Sugihartini, 2020). Less ointment base allows the environment to be less humid and the oxygen pressure in the wound tissue will decrease so that the wound healing process will be hampered (Cahya *et al.*, 2020). The high viscosity causes the spreadability of the ointment to be lower so that the ointment will be more difficult to apply to the skin and result in the absorption of the active substance being ineffective (Afianti and Murrkmiyadi, 2015; Pramesti, 2017; Puspita *et al.*, 2021).

Table 1. Number of fibroblast cells and epidermal thickness in the treatment groups

Treatment group	Fibroblast cells	Epidermal thickness (µm)
C-	50,00 ± 6,481 ^a	11,51 ± 1,47 ^a
C+	112,50 ± 4,655 ^c	39,66 ± 6,86 ^b
T1	80,50 ± 14,978 ^b	41,61 ± 1,39 ^b
T2	62,25 ± 11,558 ^{ab}	54,99 ± 4,72 ^c
T3	78,00 ± 13,736 ^b	38,43 ± 5,44 ^b

^{abc}superscript indicates significant differences ($p < 0,05$) between treatment groups.

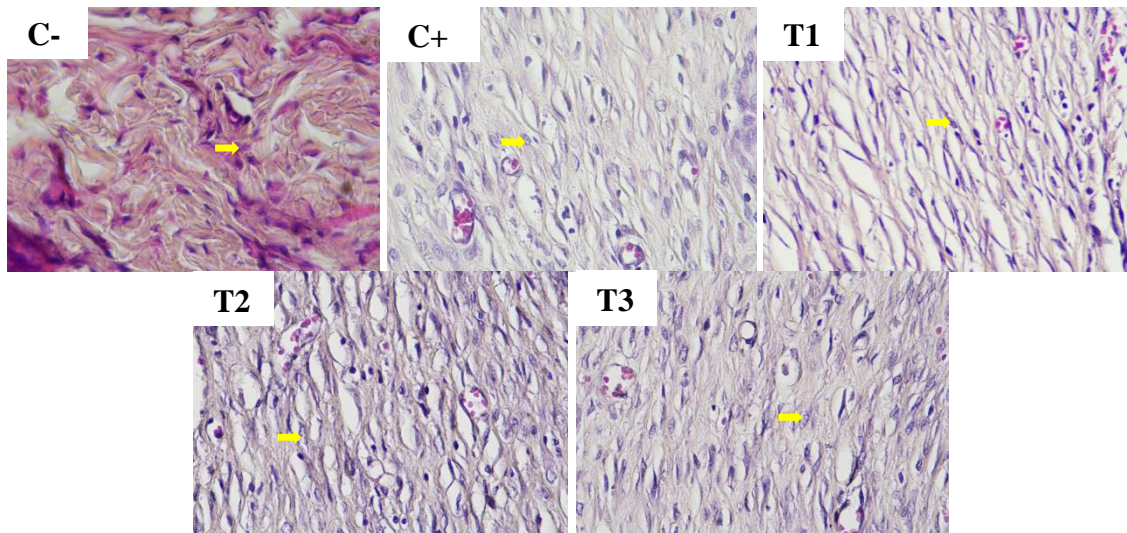


Figure 1. Histopathology of fibroblast cells in rats skin tissue using HE staining.

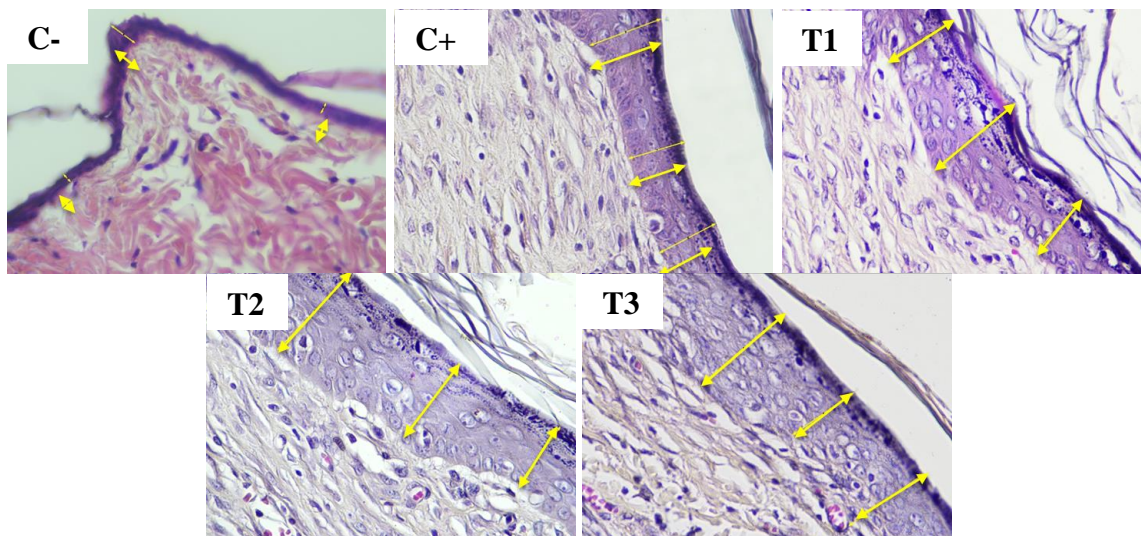


Figure 2. Histopathology of epidermal thickness in rats skin tissue using HE staining.

Group T2 with oregano extract ointment therapy with a 6% concentration was an effective therapy in helping accelerate the proliferation phase of the epidermis because it showed the thickest thickness of the epidermis. Oregano extract contains flavonoids, alkaloids, tannins, and saponins. The flavonoid content influences fibroblast proliferation by triggering the synthesis of the growth factor Keratinocyte Growth Factor

(KGF) (Pramesti, 2017). Alkaloid compounds will speed up the re-epithelialization phase by increasing the formation of epithelial cells in the epidermis. Saponin can play a role in increasing epidermal cell proliferation by synthesizing TGF-β1 and VEGF which act as growth factors and are responsible for the re-epithelialization phase (Hamid *et al.*, 2022). Meanwhile, tannins can stimulate the proliferation of epithelial cells in the

wound healing phase and help the migration of fibroblasts into the wound and encourage cellular collagen synthesis (Al Ghifari, 2021).

CONCLUSION

Therapeutic application of oregano extract ointment with a concentration of 6% was the optimal concentration in accelerating the proliferation phase in the healing process of excision wounds in albino rats by reducing the number of fibroblast cells and being able to increase the thickness of the skin epidermis.

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