OINTMENT OF *Eupatorium odoratum* L. EXTRACT PROMOTES BURN WOUND HEALING IN MALE ALBINO MICE

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ABSTRACT

*Eupatorium odoratum* L. is traditionally used to treat open wounds. Aim of this study was to investigate the healing activity of ointment containing extract of *E. odoratum* in burns induced in male albino mice. Mice were divided into 5 groups, all were induced for burn wound using a heat stamp in temperature 80°C for 20 minutes. Group I was treated with ointment base (control), group II, III and IV were treated with extract ointment in concentration of 5%, 10%, and 20% w/w respectively, and the last group V was treated with ointment reference. Observations were made during 21 days exactly on the 7th, 14th, and 21st day, which included parameters: percentage of healed area, epithelialization time and collagen scores. The result showed that on 14th day, mean of healed area in group III (75.89%±7.76%) and IV (76.29%±6.981%) were significantly higher than other groups (P<0.05). On the 14th and 21th day, collagen scores in groups III (2.33±0.577 and 2.67±0.577) and IV (2.67±0.577 and 2.33±0.577) are equal with reference group, these score are significantly higher than in groups I and II (P<0.05). Meanwhile, though epithelialization time in group III and IV (both 14.33 days±0.577days) are more quickly than others, statistical analysis showed that there is no significant difference on time between all groups (P>0.05).

Keywords: *Eupatorium odoratum* L, burns, healed area, epithelialization time, collagen scores

INTRODUCTION

Burn is one of the most common injuries experienced by humans. Burns wound are able to totally destroy the integrity of the skin. Burns can cause discomfort and death, particularly in patients older than 40 years (Mantle, *et al*., 2002).

In Indonesia, approximately 2.5 million people suffered burns injury each year. However, the handling of burn wound is less than optimal, and the limited options of medication for topical treatment for burns resulting in continued high prevalence of complications in burn patients. Improper handling of the burns will cause interference in the healing of burns and causing injury for longer time (Annan and Houghton, 2007).

One of the plants that are traditionally used to treat wounds is *Eupatorium odoratum* L. This plant is known as traditional name kirinyuh in west Sumatera. Part of *E. odoratum* used in traditional medicine are leaves containing several major compounds such as tannins, flavonoids, saponins, and steroids. Previous studies showed that the leaf *E. odoratum* have a numbers of pharmacological activities which are important in the healing process of burns such as antioxidant activity (Amatya and Tuladhar, 2011), anti-inflammatory and membrane stabilization (Umukoro and Ashorobi, 2006), antibacterial (Hasnawati and Prawita, 2010), analgesic and immune modulator (Chakraborty *et al*., 2010). In addition, preliminary study done to investigate activity of ethanol extract of *E. odoratum* leaves for treatment of burns on male albino mice showed that the ethanol extract had burn healing effect based on assessed diameter reduction of burns (Fitrianda, *et al*., 2012).

This study was conducted to investigate the burns healing effect of *E. odoratum* extract in ointment dosage form. Ointment is one of the most appropriate dosage form for the treatment of burns due to its properties including protects wound from bacterial infection, prevents heat loss due to evaporation and retains moisture in the wound area, which is very important for skin epithelialization process. Activities of burn
healing was assessed on several parameters including the percentage of healed area of burns, the time required for the formation of new epithelial or for the burns completely healed (epithelialization time), and the formation of collagen fibers.

MATERIALS AND METHODS

Plant materials

The plant sample used in this study was leaves of E. odoratam taken in the South Coast region, of west Sumatra. Identification of samples was done at the Herbarium of the Department of Biology, University of Andalas.

Preparation of ethanol extract

As much as 1 kg of chopped sample was macerated with 70% alcohol for five days and concentrated. The yield was 15.62%.

Preparation of E. odoratum extract ointment

Ointment was made in the base of hydrocarbons. Cera alba (200 mg) was melted by heating, prophyl paraben (1 mg) and alpha tocopherol (0.1 mg) were then added in to melted cera alba. Ethanol extract was properly weighted according to the desired concentration, which were 500 mg, 1 g and 2 g, for the ointment concentration of 5%, 10% and 20% respectively. These extract were added to the mixture in a mortar, and vaseline was finally added to reach 10 grams of ointment and crushed to form a homogeneous ointment.

Animals

To assess the potential effectiveness of E. odoratam extract ointment in healing burn, burns model by Nayak et al. (2008) was used. Experimental animals used were 60 male albino mice having body weight 20-25 grams. Mice were acclimatized for 10 days. Healthy animals which showed no fluctuation on weight more than 10 % and no visual symptoms of the disease during acclimatization were counted in for experiment. Animals were weighed and divided into 5 groups as follows: group I was burns induced and treated with ointment base (control), group II, III and IV were burns induced and treated with ointment in concentration 5%, 10%, and 20% w/w respectively, and the last group V was burns induced and treated with reference ointment. Each group consisted of 12 mice.

Burn wound model

Hair in the back of animals was discarded by using hair loss cream. Furthermore, animals were anaesthetized using ether. Burns were made by using 1.5 cm diameter metal stamp which was heated in hot water to a temperature of 85°C. This metal attached to the back of mice for 20 seconds until it resulted in circular burns. Ointment base, extract ointment, and reference ointment were applied 3 times daily in burns starting immediately after the induction of the burn until there is perfect epithelialization (Nayak, et al., 2008). Parameters measured on the model burns were:

**Percentage of healed area**

Percentage of healed area of burns was measured on the 7th and 14th day until the wound was completely healed. The percentage of burn wound healing was calculated by the formula:

\[
\text{% of healed area} = \left( \frac{\text{the size of healed area}}{\text{The size of initial burn area}} \right) \times 100
\]

**Epithelialization time**

This parameter is the time required for the formation of new epithelial which perfectly cover the burns. In this experiment, epithelialization time was recorded as the time when scab tissue off from the wound without leaving residual excision in wound area.

**Histopathology**

Histopathology observations were done on the 7th, 14th, and 21st. Samples of wound tissue were taken 0.3 cm from the edge of the initial injury. This tissue was soaked with 10% formalin, and then retrieved vertical slices and stained with haematoxylin and eosin. The histological preparations subsequently observed under a microscope and scored by the following criteria:

0 if no collagen fibers appear, 1 if collagen fibers spread thin or slightly, 2 if moderate collagen fibers appear and showed unification
and 3 if collagen fibers spread a lot of and perfectly bound.

**Data analysis**

Values obtained from each parameter was calculated as the mean ± standard deviation (SD). The significance of differences in average values as a result of extract ointment treatment against the control group was analyzed using one-way ANOVA using SPSS 17. P < 0.05 was considered as significant difference. Value of P < 0.05 was further analyzed by Duncan's test in order to see the significance of the mean difference caused by different treatment.

**RESULT AND DISCUSSION**

Epithelialization time is the time required for the formation of new epithelial tissue so that the wound is closed completely. Observation of these parameters was done visually by noting the day when scab tissue off from the wound without leaving residual excision in wound area. Epithelialization time shows the time when the wound has healed perfectly regardless of the tissue below the epidermis. Mean epithelialization time in group III and IV was shorter than another group. It means that epithelial formation in group treated with 10% and 20% extract ointment was occurs in faster time than group treated with ointment base, 5% extract ointment and reference ointment, although statistical analysis revealed no significance different on mean epithelialization time among these groups (P>0.05).

Percentage of healed area is a relative comparison between the healed area with extensive burns early in experimental animals. Higher value of this parameter means that the more effective the treatment given to improve the healing of burns. On 7th day, there is no significant difference of the percentage of burn wound healing in each group. This is shown by the results of statistical analysis using one-way ANOVA test that resulted in significance value > 0.05. But, on 14th day, it showed that the percentage of healed area in group III and IV were significantly higher than another groups (P<0.05). It means the treatment using ointment of extract in concentration 10% and 20% gave significant better healing effect on burns compare to control, 5% ointment, and even reference ointment.
On 7\textsuperscript{th} day, collagen fibers in all groups were seen slightly. Statistical analysis showed that collagen density scores did not differ significantly in any group (P>0.05). On the 14\textsuperscript{th} day, statistical analysis to collagen scores indicated that group I and II had significant lower score than group III, IV and V. At this observation point, collagen fibers in group I and II were seen slightly, while groups III, IV and V had thin fibers and began to form a compact tissue. On 21\textsuperscript{st} day, thin collagen fibers in groups I and II seems spread, while collagen fibers in groups III, IV and V were seen to spread and unite. Analysis of these data showed that collagen score in group III, IV and V were significantly higher than group I and II (P<0.05).
Previously, collagens were thought to function only as a structural support; however, it is now evident that collagen and collagen-derived fragments control many cellular functions, including cell shape and differentiation, migration, and synthesis of a number of proteins. Findings suggest that cell contact with precise extracellular matrix molecules influence cell behavior by regulating the quantity and quality of matrix deposition. Type I collagen is the most abundant structural component of the dermal matrix; migrating keratinocytes likely interact with this protein. Collagenase (via formation of gelatin) may aid in dissociating keratinocytes from collagen-rich matrix and thereby promote efficient migration over the dermal and provisional matrices. Cellular functions are regulated by the ECM. The information provided by ECM macromolecules is processed and transduced into the cells by specialized cell surface receptors. Evidence demonstrates that the receptors play a major function in contraction of wounds, migration of epithelial cells, collagen deposition, and induction of matrix-degrading collagenase. Although keratinocytes will adhere to denatured collagen (gelatin), collagenase production is not turned on in response to this substrate. Keratinocytes have been known to recognize and migrate on Type I collagen substratum, resulting in enhanced collagenase production. Collagen plays a key role in each phase of wound healing (Brett, 2008). Type I collagen is the most abundant type of collagen in normal dermis (approximately 80% to 90%). During the early stages of wound healing, fibroblasts actively produce type III collagen, which may account for 30% of the collagen in a healing wound. By week 2, type I collagen again becomes the principal collagen produced by fibroblasts. During remodeling, type III collagen is replaced by type I collagen to restore the normal dermal collagen composition (Hsu and Mustoe, 2010).

**SUMMARY**

Ointment of *Eupatorium odoratum* extract in concentration 10% and 20% could promote burn wound healing inducted in male albino mice, especially in application for minimal 14 days. We suggested that this activity was partly mediated by its ability to increasing collagen fiber in burned area.

**REFERENCES**


Nayak, B.S., S.S. Raju and A. Ramsubhag, 2008, investigation of wound heating


