IN VIVO WOUND HEALING ACTIVITY OF MELALEUCA ESSENTIAL OIL-BASED CREAM

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ABSTRACT

Skin injury or wound is a normal event occurs in living organisms. In normal hygiene environment, wound would achieve full recovery within 14 days. Wound may be vulnerable to bacterial infection if not treated immediately, thus delaying the healing activity. Exisiting plant-derived medicines in the form of essential oils have shown encouraging effect in wound treatment. However, to our knowledge, there is limited report documented on the wound healing activity treated with melaleuca essential oil. In this study, we wish to determine the in vivo wound healing activity following topical application of melaleuca essential oil using murine model. Full thickness of mouse skin was excised and the wounded area was topically applied either with melaleuca essential oil-based cream, silver sulphadiazine (positive control) or cream base (negative control) for 10 days post-wounding. The percentage of wound contraction monitored by digital photographs and measured by using ImageJ software. It was observed that the mice treated with melaleuca essential oil showed significant acceleration healing activity as compared to the other groups as they achieved complete wound closure within 10 days post-excision. Future works focus on the combination of essential oils and the histological analysis of wound site at tissue level.

Keywords: Wound healing, wound contraction, essential oil, melaleuca

1. INTRODUCTION

Wound is an injury of the skin due to cut, tear or trauma that disrupt or damage the skin integrity. Wound healing is a complex biological process involving few stages including inflammatory phase, proliferative phase and remodelling of skin [1]. Natural remedies or herbal medicines have widely been used as wound healing agent due to its potential in wound care with optimal and faster healing property. These herbal remedies have been found to be effectively involved in the disinfection, removal of damaged tissues from wound site along with providing a moist environment as an effort to provide a conducive environment for natural wound healing [2]. It is common practice that plant-derived medicines come in the form of plant extract or essential oil (EO). Extensive researches have been done in discovering their role and potency in wound healing management, at which EOs are the current attention because of their pleasantness and inexpensiveness. In addition, a study demonstrated a faster absorption of EO into the blood stream following topical application of EOs on skin, which is within 10-30 minutes [3].

Melaleuca alternifolia or commonly known as tea tree plant is an Australian native plant that can be found in South Wales and Queensland. Notably, melaleuca EO and its main component, Terpinen-4-ol obtained from steam distillation of leaves or branches of this plant is widely and effectively used in dermatology to treat skin diseases and infections [4]. The antioxidant, anti-inflammatory and antimicrobial properties

possessed by melaleuca contribute to wound renewal capacity as well [5]. For example, melaleuca EO has shown its effectiveness towards strains of *Staphylococcus aureus* isolated from normal wounds, surgical wounds as well as on methicillin-resistant and sensitive bacteria (MRSA and MSSA) *in vitro* [6]. Meanwhile, in vivo study revealed its antifungal activity in a murine oral candidiasis model [7]. Similar to tap water, melaleuca EO accelerated the cooling and healing process of burn wound regardless immediate or delayed application [8].

However, to the best of our knowledge, there is limited report documented for the rate of wound healing activity treated with Melaleuca EO in mice model. The present study was conducted to determine and visualize the wound closure rate following topical application of melaleuca EO on excised wound model as well as to evaluate the epithelial gap on the wound site by H&E staining.

2. MATERIALS AND METHODS

2.1 Materials

Coconut oil, avocado oil, olive oil, beeswax and shea butter were purchased from Pure Nature, Auckland, New Zealand (http://purenature.co.nz/). Melaleuca EO was obtained from doTERRA International LLC, Utah, USA, while silver sulphadiazine was acquired from Ministry of Health, Malaysia. Healthy male Imprinting Control Region (ICR) mice (6-8 weeks old, 20-30g) were purchased from Universiti Kebangsaan Malaysia.

2.2 Preparation of topical ointment

A mixture of 38% w/w coconut oil, 21% w/w avocado oil, 21% w/w olive oil, 15% w/w beeswax and 5% w/w shea butter were melted at 40^{o}C and mixed well. The mixture was cooled down at room temperature until it reached 25^{o}C before adding 0.25ml EO of melaleuca. Topical ointment without the addition of EO was used as negative control (cream base).

2.3 Wound healing assays

All experimental procedures involved in this research is according to the approved protocols by UKM Animal Ethics Committee. A total of 16 healthy ICR mice were randomly assigned into four different groups; experimental, positive and negative control groups and test group with four mice each group.

The back of the mice were shaved and applied with depilatory cream to remove any remaining hair. Then, full-thickness excisional wounds were made using sterile 5-mm biopsy punch by picking up a fold of the skin [9]. One wound was generated on each side of the midline [10, 11]. Following excision, melaeuca EO-based cream, silver sulphadiazine (SSD) or cream base (CB) was applied gently onto the wound and this step was repeated daily until the end of timeline where mouse was healed completely. Mice in control group were left untreated (UN). Digital photographs were taken for each wound every day. Percentage of wound contraction was measured by calculating the area of wound using ImageJ software.

2.4 Statistical analysis

Student *t*-test was used to compare the significant difference between treatments. All statistical analysis was performed by GraphPad Prism version 7 software. Values were represented by mean ± S.E.M.

3. RESULTS AND DISCUSSION

3.1 Melaleuca EO-based cream accelerates wound closure

The wound healing progress was monitored throughout the experiment and is presented in Figure 1. Figure 1A showed the representative images of wound area following topical application of melaeuca EO-based cream, SSD and CB as well as untreated wound. From the images, it was clearly observed that wound treated with melaleuca EO-based cream reduced consistently and almost reached complete wound closure at day 10 post-wounding. The representation of wound contraction rate in Figure 1B supports the positive effect demonstrated by melaleuca EO-based cream. Partial wound closure was observed on wound treated with melaleuca EO-based cream at day 6 post-wounding (57.5%). More advanced wound closure was observed at day 8 (80.6%), at which wounds in control groups were not closed after the same period of time (CB; 67.6%, SSD; 68.2%, UN; 69.2%). Overall, the open wound area was significantly larger in control groups compared to melaleuca EO-based cream treated mice (*P<0.05 and **P<0.01). This favourable progression of skin renewal may be due to the anti-inflammatory, antioxidant and antimicrobial properties owned by melaeuca [12, 13].

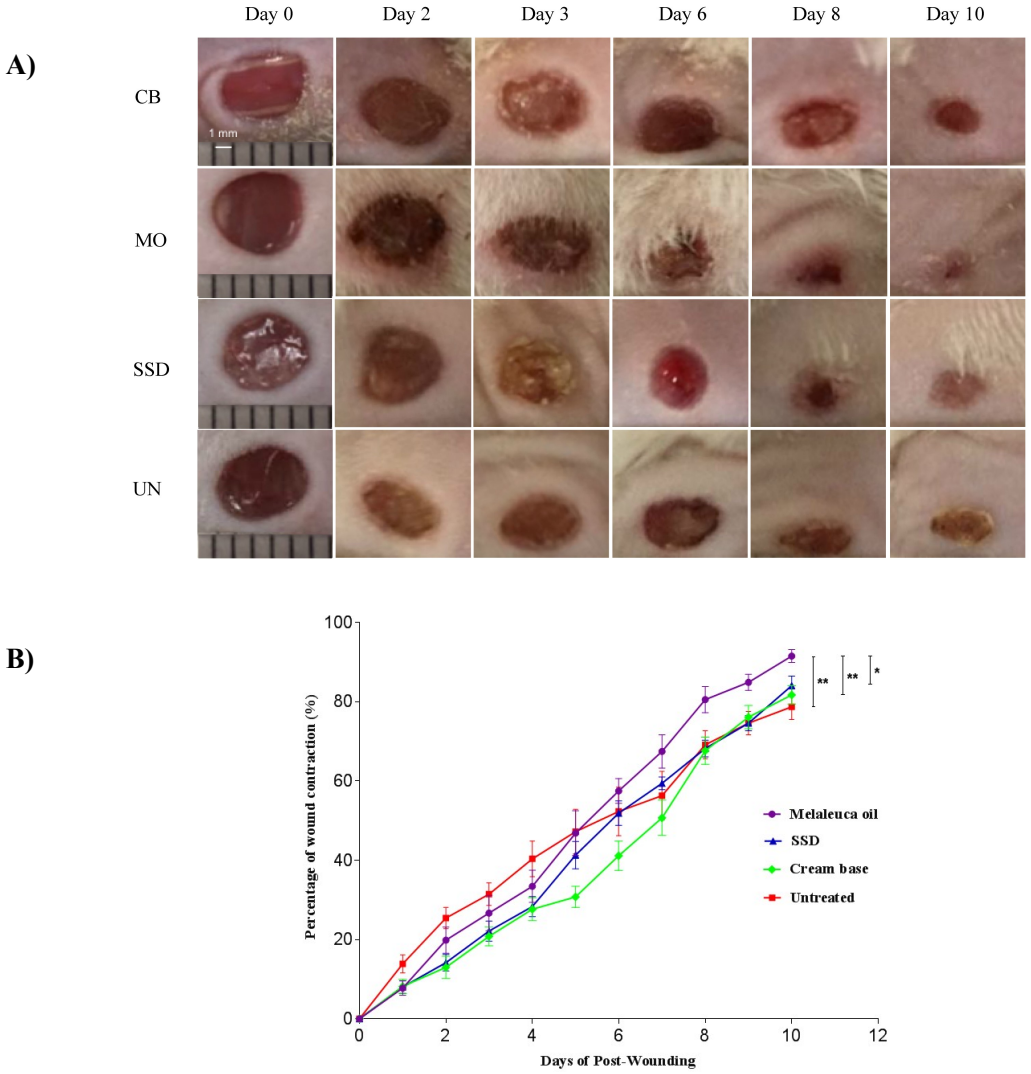

Figure 1. A) Excisional wound of group of CB, Melaleuca EO-based cream (MO), SSD and UN. **B)** Wound closure displayed as percentage as wound contraction. Values are expressed as mean ± SEM. n = 3 or 4 mice per group, *P<0.05 and **P<0.01

4. CONCLUSION

Melaleuca EO-based cream accelerates wound healing activity in excised wound model, suggesting it as an effective wound healing agent. However, future works are needed to evaluate the healing activity at tissue level including the tissue remodelling, re-ephithelization and collagen deposition to better understand the healing capacity of melaleuca EO. Combination of different EO in treating wound at pre-clinical setting could also be an important aspect for future study.

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