Wound healing effect of Rosemary and Chamomile combination in rat

Amir Hossein Abdolghaffari[1], Parvin Mahdaviani[1], Saeed Fallah-Bonekohal[1,2], Seyedeh-Farnaz Ghasemi-Niri[2], Reza Hagiaghaei[3], Seyed-Mehdi Banan-Khojaste[2], Maryam Baeeri[1], Mohammad Abdollahi[1]

[1]Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran
[2]Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran
[3]Department of Pharmacology, Institute of Medicinal Plants, ACECR, Tehran, Iran

Correspondence: Mohammad Abdollahi, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran, Email: mohammad.abdollahi@utoronto.ca

Summary

The purpose of this study was to evaluate healing effect of *Rosemarinus officinalis's* extract (ROE) *and Matricaria chamomilla's* extract (MCE) and their combination (ROE-MCE) on experimental cutaneous burn injury.
Animals were separated into four groups to receive extracts control, ROE or MCE and the combination in a cream base three times daily for 15 days starting 12 hour post induction of burn injury using a device to produce 200ºC heat. The wound image was captured on a daily basis up to 15 days and then evaluated by Image Mixel software. Samples from the wound area were obtained on the end of treatment for histopathology examination.
The percentage of the wound healing in the ROE, MCE, ROE-MCE, and control groups were 48.2, 56.5, 88.5, and 9.8 indicating that combination group was much effective than other groups. Histological examination showed that re-epithelialization of wound area was much better when combination therapy was used. The results of this study raise the possibility of potential efficacy of ROE-MCE combination in accelerating cutaneous burn injury.
Key words: healing, *Rosmarinus oficinalis*, *Matricaria chomomilla*, wound, burn injury

Introduction

One of the severe injuries that caused by burn wound is acute cutaneous injury (1). Following a burn injury, biologic responses slightly result in increasing numerous chemical mediators, such as cytokines, type II phospholipase A2, platelet-activating factor, eicosanoids, polymorphonuclear leukocyte elastase, complement, endothelin-1, thrombomodulin, nitric oxide, and adhesion molecules, in the acute phase of burn injury. On the other hand, infection increases levels of chemical mediators rapidly and affects the intensity of illness (2-8). Repression of infection is an important factor for rating of burn-wound healing. Healing of burn injuries is a complex, well-orchestrated physiological event involving a series of dynamic events, including formation of fibrin clot, inflammatory response, tissue granulation (re-epithelialisation and angiogenesis), matrix formation, and remodeling (9).

Matricaria chamomilla (chamomile) is an herb with beneficial healing effect on skin disorders such as psoriasis, eczema and acne, and especially wound (10-15). Traditionally, Chamomile is thought to have benefits as a sedative, spasmolytic, and anti-inflammatory agent (16). On the other hand, *Rosemarinus officinalis* (rosemary) is a plant with reported antioxidant activity. A number of components have been identified responsible for the antioxidant property of rosemary. The main antioxidant effect is attributed to three phenolic diterpenes including carnosic acid, carnosol and rosmarinic acid (17). Regarding antioxidant property of rosemary, it seems useful for burn wound healing.
In the present study, we examined the effect of ROE and MCE and combination of them on acute cutaneous heat-induced burn injury in rat.

Materials and methods

Extractions

The aerial parts of rosemary were collected from the Institute of Medicine of Plants-ACECR in NOV 2009 and were air-dried at room temperature. Extract was prepared using 100 g dried and powdered aerial parts of rosemary with 95% methanol solution and then the methanol extract was evaporated under reduced pressure (20 g dry weight corresponding to 9.4 %). The dry chomomile flowers were obtained from the Institute of Medicinal Plants (ACECR) in NOV 2009 and dried at room temperature. The chomomile dried powder was extracted by methanol 95% by percolation and then methanol was evaporated (15 g dry weight corresponding to 9.4%). The extracts were dissolved in the cream basis that is containing 15% net extracts. Cream was made by mixing of 15g dry weight of ROE or MCE in 85 g eucerin. In the combination form, 7.5 g of each extract was dissolved in 85 g eucerin.

Experimental model and method

Rats weighting 250-300 g from animal house of TUMS were accommodated in standards temperature (25 ºC) and light (12 h light, 12 h dark). A total of 16 rats were used with equal numbers being assigned to each of four groups including control, ROE, MCE, and the combination (ROE-MCE) groups. On the first day, animals were anaesthetized by injection of pentobarbital (50 mg/kg, IP) and then a full-thickness circular 15-20 mm diameter burn wound was created using an electrical heater (200 ºC for 15-20 sec) on dorsal part of the shaved rats. The underlying skin was cleaned with normal saline. Animals bearing third-degree burn wounds were distributed into four groups each containing 5 animals. Treatment groups were dressed three times daily with ROE, MCE, and ROE-MCE while controls received only eucerin® for 14 days. Before taking picture by a digital camera, the wounds were flushed by sterile saline to remove debris and to clean the wound area. The distance of camera to wound was fixed by a fixer set to standardize scoring according to wound area and appearance of new fresh epithelium. The captured images were examined by Image Mixle software to evaluate wound area (WA). Improvement symptoms of dermal burn injury was proved by degree of wound improvement, proliferated tissue, and formation of epithelium in disturbed dermis. The improvement percentage (IP) of wound healing was calculated according to the following formula:

$$\text{Healing rate} = \frac{(1\text{th day WA}) - (15\text{th day WA})}{1\text{th day WA}}$$

Histochemical analysis

Granulated tissue was collected on the 15th day and preserved in 10% buffered formalin. Series of 3-4 µm thickness sections were prepared and stained with haematoxylin and eosin and photographed under 100 x magnifications (18, 19).

Statistical Analyses

Results were analyzed by SPSS, version 16. One-way ANOVA followed by Tukey's posthoc tests were used to evaluate changes between groups. A *P*-value of less than 0.05 was considered significant.

Results

Degree of wound improvement

Data are shown in Figures 1. The percentage of wound healing in the groups of controls, ROE, CME, and RME-CME were 9.8, 48.2, 58.5, and 88.5, respectively. The rate of healing by all treatment groups was significantly higher than that of controls. Meanwhile the combination of RME and MCE was significantly more effective than that of each alone.

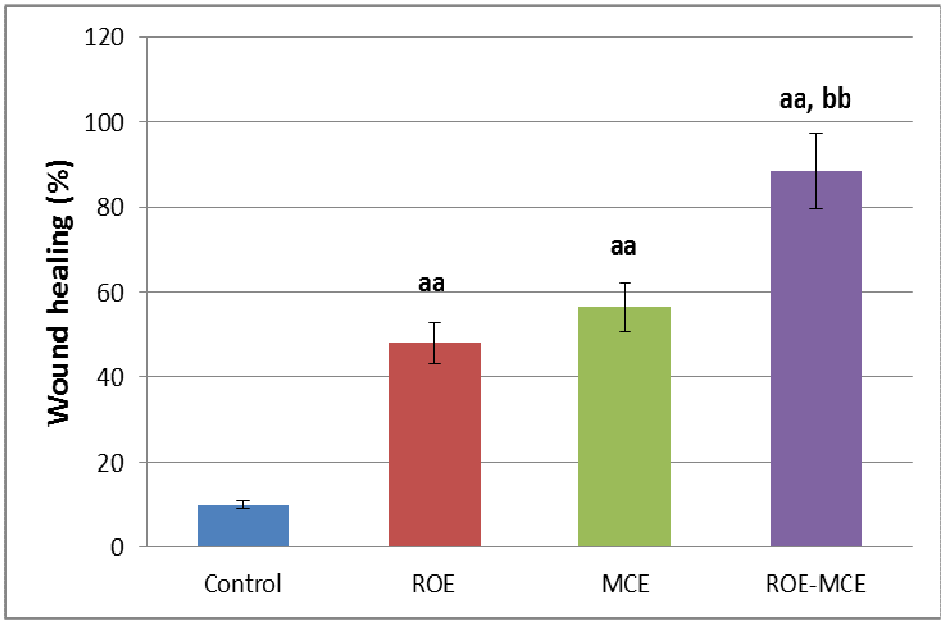

Figure 1. Rate of wound healing in various groups.
Groups included *Rosemarinus officinalis's* extract (ROE), *Matricaria chamomilla's* extract (MCE), and their combination (ROE-MCE). The captured images were examined by Image Mixle software to evaluate wound area (WA). The healing rate was calculated on 15th day of treatment according to the formula: (1th day WA)-(15th day WA))/(1th day WA)
[aa]Significantly different from control at P<0.001; [bb]Significantly different from ROE and MCE at P<0.001.

Macroscopic pictures of wound area on the days 1, 7, and 15 of treatment in various groups are shown in Figure 2.

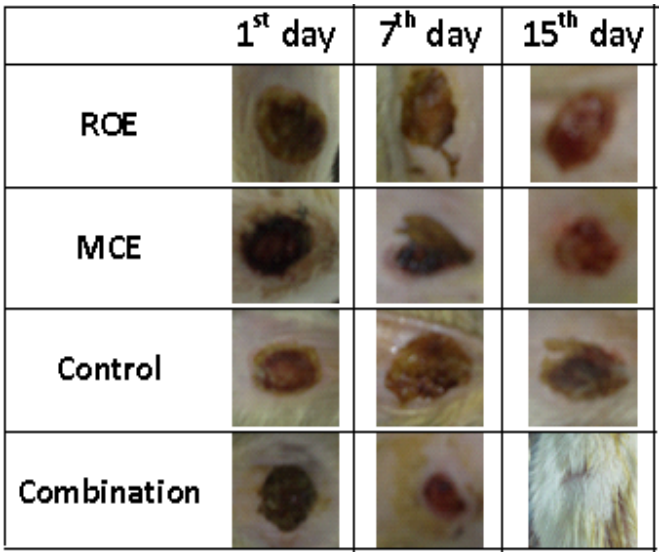

Figure 2. Macroscopic pictures of wound area in various groups.
Groups included *Rosemarinus officinalis's* extract (ROE), *Matricaria chamomilla's* extract (MCE), and their combination (ROE-MCE). The images were captured by a digital camera on the days 1, 7, and 15 of treatment.

Histopathological study
Data are shown in Figure 3. Improvement in treatment groups in terms of tissue proliferation and formation of epithelium was evident in comparison to treatment groups. The rate of epithelium formation in RME-CME group was significantly higher than that of each alone.

Discussion

Wound healing is quite a complicated process involving epidermal regeneration, fibroblast proliferation, neovascularization and synthesis. Although there have been some treatments, the best treatment still remains complicated. Many investigations have considered acceleration and whether the duration of wound healing could be shortened. In many previous studies, natural products for the treatment of burn wounds have been used, but these were mainly aimed at controlling infectious (20,21). Anti-inflammatory activity of certain natural products play a main role in the healing of burn wound (22). Constituents of MCE include terpenoids, flavonoids, coumarins, and mainly chamazulene, apigenin, and bisabolol (23). Drugs with anti-inflammatory, antibacterial or antioxidant properties may be good candidate for burn wound healing like MCE that has all of these properties (13, 14, 15). On the other hand, RME is found to have anti microbial, anti bacterial, anti fungal (24) and anti cancer effects (25). α-Pinene, bornyl acetate, camphor, and 1,8-cineole are anti microbial compounds in the RME (26).

Many compounds have been separated from RME containing diterpenes, steroids, and triterpenes. Two phenolic diterpenes that have shown antioxidant effects are carnosic acid and carnosol (15, 28-30). One of the factor that impair the healing process is free radicals that are generated at the site of injury causing damage to cellular membranes, nucleotides, proteins and lipids (31). Therefore it would not be surprising if it would be concluded that wound healing effects of RME comes mainly from its antioxidant potential (32-33). The present study showed a marked synergistic effect of RME and MCE in the wound healing, thus this mixture can be a good candidate for achieving faster healing of wounds, without complications.

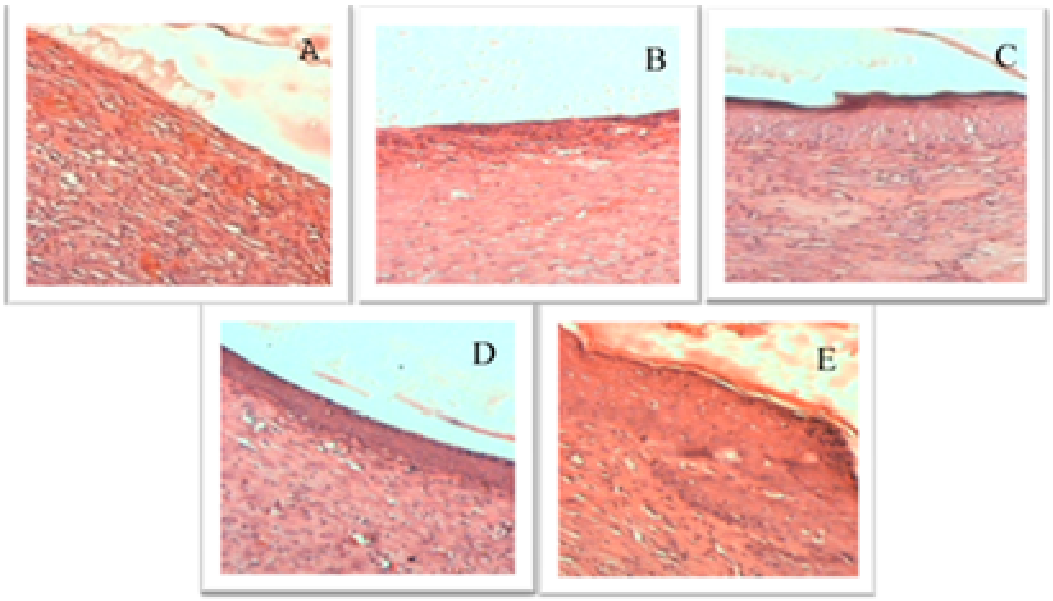

Figure 3. Histopathology of derm in various groups.
A: Histogram from derms of animals treated by only eucerin as the cream base. Epithelium and tissue proliferation are not seen (H&E ×100). **B:** Histogram from derms of animals treated by MCE. Comparing with figure A, in the disturbed derm, formation of epithelium and tissue proliferation under the epithelium are seen (H&E ×100). **C:** Histogram from derms of animals treated by ROE. Comparing with A, in the disturbed derm, formation of epithelium and tissue proliferation under the epithelium are seen (H&E ×100). **D:** Histogram from derms of animals treated by Combination of MCE-ROE. Comparing with A, in the disturbed derm, epithelium is completely formed and tissue proliferation under the epithelium is evident (H&E ×100). **E**. Histogram from not burned derms. Comparing with A, the epithelium and stratum corneum are clear (H&E ×100)

Acknowledgement

This study was partly supported by a grant from TUMS.

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