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Healing Effect of Sesame Ointment on Second-degree Burn Wound in Rats

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Abstract

Background: Wound healing requires processes like cell migration, proliferation of extracellular matrix deposition, remodeling and angiogenesis. Also, anti-oxidative and anti-inflammatory reactions play key roles in the progression of wound healing. Previous studies showed that sesame oil has anti-inflammatory, antimicrobial and antioxidant activities. In addition, it is shown that sesame oil stimulates fibroblast production in vitro and in skin wounds. Therefore, in this study we investigated the effects of sesame ointment on the process of second-degree burn wound healing in rats model according to stereological parameters. **Materials and Methods:** In this experimental study, forty male Wistar rats (200±20 g) were randomly divided into four groups (n=10): ointment-base treated group (vehicle), silver sulfadiazine (SDD) treated group, sesame treated group (E1) and the control group which received no treatment. A 2×3 cm2 standard second-degree burn wound was induced on the posterior surface of animal's neck under general anesthesia. After sacrifice, the animal's skin sample was fixed in buffered formaldehyde for stereological evaluations. The data was analyzed by SPSS statistical software (version 14.0). P<0.05 was considered statistically significant.**Results:** According to our results, the mean of reduction in wound areas, volume density of collagen bundles and hair folicules, fibroblast populations, length density of vessels in E1 group was significantly higher than control group (P<0.05). The differences between E1 and SSD were not statistically noticeable regarding the stereological parameters. **Conclusion:** According to stereological analysis, administration of sesame ointment showed the ability to improve wound healing process and tissue regeneration in the treatment of skin damages.**[GMJ.2016;5(2):56-62]**

Keywords: Wound Healing; Sesame; Stereology; Burn Wound; Second Degree

Introduction

There are various kinds of wounds, the most important of which are burn wounds due to their susceptibility to infections because of vascular necrotic tissue. Loss of epidermal integrity of the skin is the main consequence of burn wound. Thermal electrical and chemical injuries are the most common causes of these wounds. The severity of burn injuries is deter-

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mined according to the depth of skin involvement and the percentage of total body surface area involved [1].
Burn wound healing of an adult skin as a complex process leading to scar formation involves three main phases; the inflammatory phase, proliferative phase and remodeling of extra cellular matrix phase. Preparing the wound for repair is the main function of the first phase [2]. Fibroblasts appear at the beginning of second phase to restore collagen lattices and angiogenesis. The final step of this phase is epithelialization involving migration, proliferation and differentiation of epithelial cells from the wound edges to resurface the defect [3]. Fibroblasts and their matrix proteins, especially collagens, are essential for tissue repair and remodeling [4]. The third phase much longer which differs from a few weeks to some years. Finally wound contraction undergoes fibroblasts responsibility occurring via migration of the wound edges toward the center that makes wound size reduced [2].
The role of each of the compartments including cell types during the mentioned phases as well as the growth factor and matrix signals present at a wound site, are now roughly understood [5]. Extracellular matrix and soluble growth factors regulate the migration of epidermal and dermal cells to the wound bed, which is critical to wound healing [6]. Many efforts have been applied by clinicians to manage wound healing through using genes, cytokines, chemokines and surgery. However, an untreated burn wound would progress often within 72 hours and makes treatment more difficult, prolongs hospital stay, increases costs and the likelihood of scarring is noticeable. Some involved mechanisms in the process are local tissue hypoperfusion, edema, prolonged inflammation, hypercoagulability, free radical damage and accumulation of cytotoxic cytokines [7]. Thus, more investigations are largely needed to understand the complex pathogenic mechanisms involved in burn wound healing and to reduce expenditure and shorten time cost related to this course.
In the burn area, two main growth factors are secreted by residual skin cells called vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) that drive the force behind wound healing [6].
Burn healing has remained challenging in modern medicine and there are a few drugs capable of accelerating wound healing. Toxicity is one of the most restrictions that prevents using compounds with promising activities against collagen synthesis or fibroblast proliferation [2].
Regarding this, plants are rich sources that can be taken advantage of. Thanks to moderate efficacy, none or less toxicity, low cost and more availability of herbal products are one of the best alternatives to survey. There are several reports in literature using herbal drugs to heal burn injuries [4, 8-13].
Sesamumindicum L. (Sesame) belongs to Pedaliaceae family. As an herbaceous annual plant, it is cultivated due to its edible seed, oil and flavorsome value [14]. Various bioactive components of the seed were reported including vital minerals, vitamins, phytosterols, polyunsaturated fatty acids, tocopherols and unique class of lignans such as sesamin and sesamolin. A group of phenylpropanoids named lignans present along with tocopherols and phytosterols and provide defense against reactive oxygen species through antioxidant characteristics [15]. Subsequently, sesame seeds as rich source of antioxidants and bioactive compounds have also been used to treat burns indicating different effects on wound healing [16]. In addition, sesame contains substances that can inhibit lipid peroxidation process, improve the supply of blood to collagen tissues, increase the fibril collagen longevity and reduce cell damages [17].
Therefore, in this study we investigated the effects of sesame ointment on the process of second-degree burn wound healing in rat models according to stereological parameters.

Materials and Methods

1. Preparation of Sesame Ointment

A 1% (w/w) sesame-ointment was prepared by the trituration method in a ceramic mortar and pestle. For this, 1 g of sesame oil (≥95%, Sigma-Aldrich) was incorporated in 100 g of the simple ointment base composed of 5% (w/w) wool fat, 5% (w/w) hard paraffin, 5%

(w/w) cetostearyl alcohol and 85% (w/w) white soft paraffin. Silver sulfadiazine (1%, w/w) ointment was obtained from Sian Darou CO., Ltd. and it was used as a standard drug. The concentrations of the sesame oil were chosen according to a previously conducted pilot study.

2. Animals and Second-degree Wound Model

Forty male Wistar rats (200±20 g, non-fasted) were randomly divided into 4 groups. Ointment-base (vehicle) treated group, Silver sulfadiazine (SSD)1% treated group, sesame 1% treated group (E1) and the control group which received no treatment.

A 2×3 cm2 standard second-degree burn wound was induced on the posterior surface of animal's neck under general anesthesia by using a method employed by Kaufman *et al*. [18]ttreatments were carried out every 24 hrs for 15 days.

Animals were sacrificed with a high dose of ether on day 15. Full thickness skin samples (1×1 cm) were provided from the wound site and were fixed in buffered formaldehyde (pH=7.2) for further procedures.

The study protocol was approved by the Medical Ethics Committee of Shiraz University of Medical Sciences and the animal care was in accordance with the related guidelines.

3. Stereological Study

Stereological study was performed according to the methods reported by Yadollah-Damavandi [19]. The area of the wound was measured every 3 days starting from day 0 by using a digital photograph and a previously reported stereological method [19]. To calibrate the magnification, a standard ruler had been set at the level of the wound in each photograph. All results were estimated by using a stereology software composed of a point grid, and by using the following formula: Area = $\sum P \times a/p$; where $\sum P$ were the total points laid on the wound area and a/p, the area is surrounded by every four crosses, was considered as the area per point (mm2) [20]. Thereafter, the wound closure rate was calculated as: wound closure rate (%) = ((area at visit 1 − area at each visit)/ area at visit 1) × 100.

Isotropic uniformly random (IUR) sections of the blocks with 5 and 15 μm thickness were created and stained with both H&E and Hedenhain's azan-trichrome stain [20].

Microscopic analyses of the dermis were performed by using a video-microscopy system made up of a microscope linked to a camera (Alpha-200; Sony™; Japan) and a flat monitor. The volume densities of the collagen bundles, vessels and hair follicles (Vv; fraction of the unit volume of the dermis which is occupied by the collagen bundles, vessels, or hair follicles) were estimated by using the stereological point counting method and the following formula: Vv(collagenr vessel or hair follicle/dermis) = P(collagen or vessel or hair follicle)/P(dermis); where P(collagen or vessel or hair follicle) is the number of points hitting the profiles of the collagen bundles, vessels, or hair follicles; P(dermis) is the number of points hitting the reference visible field (dermis).

The numerical density (Nv; number of cells per unit volume of the dermis) of the fibroblasts was estimated by employing 15 μm slides, "optical dissector" method [2], and the formula: $Nv = \sum Q / \sum A \times h$; where "$\sum Q$" is the number of nuclei coming into focus in the dissector height, "ΣA" is the total area of the counting frame in all microscopic fields, and "h" is the height of dissector within which the counting is done. The upper and the lower 5 μm were considered "area of safety".

4. Statistical Data Analysis

Results were reported as mean and standard deviation (mean±SD). SPSS statistical software (v.14.0) was used to do the statistical comparisons between the groups. The statistical analyses were carried out by employing Kruskal Wallis and Mann Whitney U tests. Furthermore, P≤0.05 was considered as statistically significant.

Results

1. Wound Area

The mean initial area of the wounds was 100.23±4.31 mm2 (range 92.71 –112.46 mm2). No significant difference was found among four groups regarding the primary wound surface area. However, the rate of wound closure in SSD(8.21%/day) and sesame (8.18%/day) treated groups were significantly higher (P<0.05) in comparison with the vehicle (5.61%/day) and the control group (5.52%/day) (Figure-1). In addition, the group treated with the ointment base revealed an insignificantly slower closure rate compared to the control group. According to Figure-1, the wound areas increase in vehicle and control groups up to day 3 and then decrease with a slope almost similar to the sesame-treated groups.

2. Fibroblast Population

The numerical densities of fibroblasts in the dermis of the E1 group was noticeably higher than those of control and vehicle groups. The numerical density of fibroblasts in E1 and SSD groups were 24.7% (P=0.018) and 54.9% (P=0.028) higher than the controls, respectively, and 38.6% (P=0.039) and 62.29% (P=0.013) higher than the vehicle group, respectively (Table-1).

3. Volume Density of Collagen Bundles and Hair Follicles

The volume densities of collagen bundles were significantly higher by 32.3% (P=0.02) and 39.8% (P<0.001) in SSD and E1 groups in comparison to that of the control group (Table-1). In contrast with the vehicle group, volume densities of the collagen bundles were significantly higher by 41.2% and 49.2% (P<0.001) in SSD and E1 groups, respectively. The volume densities of hair follicles in SSD and E1 groups were significantly higher compared to the vehicle group (P=0.032 and P=0.02, respectively) and the control group (P=0.01 and P=0.019, respectively).

4. Volume Density, Length Density and Diameter of Vessels

The length densities of vessels in SSD and sesame treated group were significantly higher compared to the vehicle group (P=0.01and P=0.001, respectively) and the control group (P=0.02 and P=0.005, respectively). As Table-1 shows, there are no considerable differences regarding the volume densities and mean diameters of the vessels among four groups.

Discussion

The mechanism of burn wound healing is a complicated network and differs from other types of wounds such as incision, laceration or chronic ulcer. Despite unclear actual mechanism of improved healing, providing necessary material for the process, increasing blood flow to burn area, decreased inflammatory response and decreasing rate of infection are the most desirable actions that we are able to launch [6].

Nowadays, understanding wound healing is much more than classifying the process to three phases: "inflammation, proliferation and maturation." This dynamic process is a

Table 1. Mean±SD of numerical density of fibroblasts (×103 per mm^3), volume densities of collagen bundles (%), hair follicles (%) and vessels (%), length density (mm/mm^3) and the mean diameter (μm) of the vessels in the dermis of wounded rats treated with SDD and sesame ointment, those treated with ointment base (vehicle) and the untreated group (Control).

Groups	Fibroblasts	Collagen	Hair	Vessels		
	Numerical density	% Volume density	% Volume density	% Volume density	Length density	Mean Diameter
Control	189.27(18.02)	49.29(1.19)	1.99(1.22)	1.98 (0.71)	16.37(4.81)	13.17(1.54)
Vehicle [a]	199.08(38.09)	46.17(5.26)	2.03(1.21)	1.83(1.28)	16.09(5.17)	12.94(1.51)
SDD [b]	276.33(100.31)*	65.23(6.22)*	5.37(1.72)*	2.03(0.91)	31.54(7.37)*	11.03(2.23)
E1 [c]	270.21(86.02)*	68.93(2.27)*	5.11(1.86)*	2.02(1.11)	33.23(9.51)*	11.01(9.87)

* P<0.05, Control group vs. vehicle group
[a] **Vehicle:** ointment-base treated group
[b] **SSD:** silver sulfadiazine group
[c] **E1:** sesame treated group

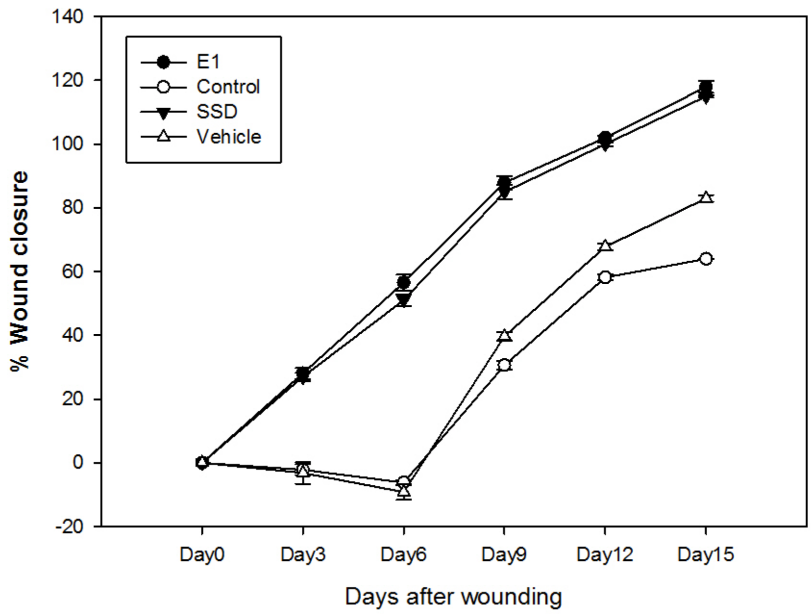

Figure 1. The effect of sesame oil on wound closure rate. Groups consisted of control, vehicle and SDD and sesame-treated rats. Each point represents mean ± SD of eight wounds. The symbol "*" shows significant difference in the SSD and sesame-treated rats compared to the control group and the vehicle group (P < 0.05).

complex series of reactions and interactions involving soluble mediators, blood cells, extracellular matrix and parenchymal cells. Our findings about new mediators are increasing each year and the more we learn about the constituents of the process, the more we find about reactions and interactions [1, 21].

Numerous studies developing more sophisticated dressings to advance healing process and diminish bacterial burden in wounds have been approached. Many researchers tried to introduce appropriate treatment methods to reduce wound infection risk and to shorten the period of treatment. So, antimicrobial agents were utilized effectively to reduce mortality rate of burns, one of which is 1% SSD. Brilliant advantages made it known as the gold standard of anti-microbial topical drugs for patients with burns [8], and its topical ointment is the most commonly prescribed medicine for partial and full-thickness burns [22].

It has been accepted that the wound repair acceleration is the best treatment method for burn wounds. Plants have an extensive potential to manage and accelerate burn wounds treatment with their antioxidant, anti-inflammatory and antimicrobial activities. Herbal cream application revealed of burn wound healing characteristics which were referred to the antioxidant, anti-inflammatory and antimicrobial activities of its phytochemical contents [23]. An ointment named Olea, as a combination of honey, olive oil and sesame is utilized in Iran for burn treatment. It was indicated that using this ointment not only prevented wound infection effectively, but also accelerated tissue repair [16].

There are contradictory reports on using sesame to heal burn wounds. Kogan *et al*. indicated that sesame oil alone has no beneficial effects on healing burn wounds in animal samples [24] while Kiran and Asad reported that sesame oil accelerated wound epithelialization up to a week and also a better contraction of the wound was observed. Therefore, as of antioxidants content among sesame seeds preventing cellular damage and facilitating tissue repair can be achieved through topical application and systemic consumption [25], tissue repair undergoes the effect of sesame oil as revealed by Valacchi *et al* [26].

Our results indicated that utilizing sesame ointment was as effective as SSD. Using sesame ointment accelerated the wound healing process and its contraction. From day 0 to 9, the healing trend was faster and the most difference was observed during the first 6 days. According to our results, numerical densities of fibroblasts in the dermis increased undergoing ointment; this means there were substances in this ointment that stimulated fibroblast proliferation. The effects of SSD and sesame ointment were statically similar. The volume densities of hair follicles in the SSD and sesame treatments were significantly higher compared to other groups. This reveals of attracting needed factors for follicle forming in the wounded area by sesame and SSD treatments. Despite inconsiderable differences regarding the volume densities and mean diameters of the vessels among four groups, the length densities of the vessels in SSD and sesame treated groups were significantly higher. Angiogenesis is a necessity to provide mediators in the wounded area and it seems sesame treatment is effective in this phenomenon.

There are various studies reporting different mechanisms of burn wound healing. Aloe vera, honey and milk ointment was shown to induce cell proliferation, increasing the wound closure rate, blood vessel counts and collagen fiber density in treated animals. It also reduced the wound secretions, inflammation and scar formation [27]. It was demonstrated that by shortening inflammation phase through receiving Argan oil twice daily, mRNA levels of TGF-β1 increased from 39.66- to 58.70-fold among treated animals [28]. This phase was also declined by secretion of cytokines and activating macrophages via chitin and sepia ink hybrid sponge treatment [29].

Conclusion

Based on our results sesame ointment may accelerate burn wound healing by shortening the inflammation phase and promoting the proliferation phase. Therefore, according to lack of toxicity, sesame utilization in order to accelerate burn wound healing is an effective and inexpensive alternative treatment for such an issue. However, accompanying this treatment with other effective materials such as ozone enhances the healing rate more significantly.

Conflict of Interest

None declared.

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