Using toasted barley in sesame oil mixture for non-surgical necrosis debridement of experimental burns in rat

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Accepted 1 December, 2013

Burns are the most damaging kind of injuries, major global public health crisis and the fourth most common types of trauma. Decision making in the treatment of burns remains a challenge despite existence of improved assessment techniques and treatment procedures. One of the most complications in healing of burn tissues is necrosis. Several medicinal plants were used to treat skin disorders as burn wounds and necrosis so far. In our traditional medicine, one of the non-surgical methods for debridement of necrotic tissues is using toasted barley in sesame oil. Animal was used, because of the lack of previous scientific research about this herbal mixture for debridement of necrotic tissue. After being anesthetized, the second-degree burns were created in rats. Animals were divided into group I, not treated (control group) and group II treated with fibrinolysin ointment 3% (positive control group). Groups III, IV and V were treated with 1, 3 and 5% w/w doses of toasted barley in sesame oil, respectively. Histopathological and clinical findings showed that treated tissues, especially at dose of 5% w/w were more debrided. Therefore, with respect to our results, this herbal based compound can be used as remedy for debridement of necrotic tissue.

Key words: Burn, debridement, necrosis, sesame oil, toasted barley.

INTRODUCTION

Skin is the most coated barrier of the body. Burns and damages to the skin due to heat, electricity or chemicals, associated with pulmonary damages, are the most important causes of morbidity, mortality and disability (Iqbal et al., 2013). Because burns have several complications and patient is involved in the long term treatment of lesion, this damage is the worst kind of trauma (Lawrence et al., 2012; Carneiro, 2002). In most countries, there are many researches on the epidemiology, etiology and consequences of burns. In children under 14 year of age, flame burns is the most common causes of burn injury with average rate of 19%, mortality rate of 6.4% and 6% outbreak (Maghsoudi et al., 2005) and children under the age of 12 are more at risk, making up 69.5% of all admissions (Scheven et al., 2012). Deep burns to skin, causes not only the epidermis
and dermis, but sometimes the under layers of dermis such as fascia muscle tissue, muscles and even bones are charred (Busuio et al., 2012). Necrotic is one of the most complications of burns. Necrotic skin creates a layer below which is a good place for growing germs and infections (Ching and Smith, 2012). The burn infection can even create septicemia, which is the major cause of burn complications and hospitalization (Wang et al., 2012). In addition, a necrotic tissue not only prevents medications to reach the wound surface but also prevent the skin grafting (Ho et al., 1998). So, the removing of necrotic tissue medically named debridement, is often necessary and is a useful way for treatment of necrotic tissue. In fact, debridement is the medical removal of dead, damaged, or infected tissue to facilitate the healing of the remaining healthy tissue. Removal methods may be surgical (Ho et al., 2012), chemical (Frank et al., 2008), autolysis (self-deigestion) (Ramundo and Gray, 2009), mechanical (Lafitte and Jones, 1989), and maggots therapy (Lepage et al., 2012). Surgical debride- ment have problems such as transfer of the patient to the operating room, anesthesia risks, surgical stress and excessive bleeding during surgery which are the causes of physician tendencies towards the non-surgical methods (Kogan et al., 2001). The aim of many researches is to find treatments that are inexpensive and easy for selectively removing the necrotic tissue (Nusbaum et al., 2012). Although synthetic drugs have favorable effects with respect to the time saving in debridement, rapid wound healing, infection prevention and patient compliance with medication, some criteria such as availability, ease of preparation, low costs and fewer side effects of herbal drugs should be considered (Saraf, 2010). The mixture of toasted barley in sesame oil, as herbal remedy, has been used for debridement necrotic burns in our traditional medicine. Because the lack of previous scientific research about the effect of this herbal mixture on debridement, this research was designed and may provide an established scientific evidence for introducing new herbal drug for debridement of necrotic burns.

MATERIALS AND METHODS
Preparation of toasted barley in sesame oil
Available commercial dried barley and sesame (Sesamum indicum plant) seed oil were prepared.

The dried barley was heated and ground to fine powder. Concentration of 1, 3 and 5% w/v of toasted barley powder in sesame oil were prepared by stirring on hot plate magnet stirrer at 40°C for 24 h for obtaining the toasted barley in sesame oil mixture as our traditional herbal medicine.

Animals
Thirty male Sprague-Dawley rats (Rattus norvegicus) weighing 250 ± 50 g were used. Animals were housed in individual cages with lighting conditions (12 h light/dark), temperature 24°C and were freely have access to water and rat food. The experimental procedure was approved by the ethics committee on animal experimentation of Bushehr University of Medical Sciences.

Inducing skin burn
Animals were subjected to anesthesia by intramuscular injection of 10% ketamine (90 mg/kg) and 2% xylazine (10 mg/kg) combination (Cesarovic et al., 2012; Kawai et al., 2011).

For creating the second-degree burn, the back shaved area was antisepsis with 1% polyvinylpyrrolidone ipidine. Then digitally controlled aluminum hot plaque of 10 mm² with temperature of 100°C was placed on the dorsal region with constant pressure for 16 s. Immediately, the analgesia with dipyrone sodium (40 mg/kg) was performed intramuscularly, being maintained for two consecutive days of oral administration of sodium dipyrone (200 mg/kg) in drinking water (Tavares et al., 2012). The approval of the second degree burn was confirmed by macroscopic, microscopic and clinical evaluations after 24 h post-burn induction. Animals were divided randomly into five groups (N=10). Group I, not treated (control group) and group II that treated with fibrinolysin ointment 3% (positive group). Groups III, IV and V were treated with doses of 1, 3 and 5% w/w of toasted barley in sesame oil, respectively. The treatments were applied topically once a day, starting from the wound induction day to the 28th day. The wounds were left undressed and evaluated daily.

Clinical signs
The clinical signs on the basis of criteria as amount, type and color of wound secretions, were done on the 5, 10 and 15 days after burn induction. The amount of secretion that represents the burn inflammation and infection was scored as heavy, moderate, scant and none. The type of secretion that represents the kind of fluid was scored as serous, serous-sanguineous, sanguineous, serous-purulent and purulent. The color of secretion that represents the tissue appearance was defined as: bright yellow, bright red, reddish, creamy and white (Kumar et al., 2006).

Histopathological assessments
Biopsies were taken on the days of 1, 14 and 28 after burn induction, by animals anesthetizing with intramuscularly injection of combination of 10% ketamine and 2% xylazine at dose of 10 mg/kg and 90 mg/kg respectively. Specimens were immediately fixed by 4% v/v formaldehyde in phosphate buffered saline (0.01 M, pH 7.2) followed by paraffin embedding. 5 µm sections preparation and haematoxylin and eosin staining (H&E). The micrographs were taken by light microscope equipped with CCD camera (Moticam pro 280, Motic Instruments Inc.) and processed using Motic image plus2 software. The histopathology parameters such as vessel and fibroblast counts and epithelial thickness were evaluated. Also, morphometric assessments of wound closure were done on the 7, 14, 21 and 28 days after burn induction.

The wound closure was calculated by the following equation:

\[
\text{Wound closure (\%)} = \left( \frac{A_0 - A_x}{A_0} \right) \times 100
\]

\(A_0\) wound area on the first day of burn induction. \(A_x\) wound area at \(x\)th day after burn induction.

Morphological assessments such as epidermis, dermis, blood vessels, cell count, and arrangement of collagen fibers were done.
**RESULTS**

**Clinical findings**

By considering the amount, type and color, a lot of secretions were found in all groups except group II and V which were treated with fibrinolyisn and 5% toasted barley in sesame oil, respectively (Table 1). The color of wound in group II and V where more reddish than other groups on the 15th day. Also, by considering the type of secretion, serous was assigned to the groups II and V on the 15th day of study (Table 1).

<table>
<thead>
<tr>
<th>Sign</th>
<th>Group</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secretion amount</td>
<td>5th day</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Heavy</td>
<td>Moderate</td>
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<tr>
<td></td>
<td>10th day</td>
<td>Scant</td>
<td>Scant</td>
<td>Moderate</td>
<td>Heavy</td>
<td>Scant</td>
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<tr>
<td></td>
<td>15th day</td>
<td>Moderate</td>
<td>None</td>
<td>Scant</td>
<td>Scant</td>
<td>None</td>
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<tr>
<td>Secretion type</td>
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<td>Seropurulant</td>
<td>Purulant</td>
<td>Sanguinos</td>
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<tr>
<td></td>
<td>10th day</td>
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<td>Serous</td>
<td>Sanguinos</td>
<td>Seropurulant</td>
<td>Serous</td>
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<tr>
<td></td>
<td>15th day</td>
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<td>No secretion</td>
<td>Serosanguinos</td>
<td>Sanguinos</td>
<td>No secretion</td>
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<tr>
<td>Wound color</td>
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<td>Reddish</td>
<td>Creamy</td>
<td>Creamy</td>
<td>Bright red</td>
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<tr>
<td></td>
<td>10th day</td>
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<td>Bright red</td>
<td>Reddish</td>
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<td></td>
<td>15th day</td>
<td>Creamy</td>
<td>Bright red</td>
<td>Reddish</td>
<td>Creamy</td>
<td>Bright red</td>
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</tbody>
</table>

*Significant value (P≤0.05), data is presented as Mean ±SD.

**Morphologic study**

Morphologic evaluations showed that the growth of epithelium thickness and rearrangements of dermal collagen in the group V was more than the other groups on the days, 14 and 28th of study (Figure 1).

**Morphometric study**

As shown in Figure 2, there is a significant decrease in the wound area (or increase in the wound closure) in groups II and V as compared to the control group on the days, 7, 14, 21 and 28th. Evaluations of debrided tissues showed less necrotic and stiffness in groups II and V (Figure 3). Also, vessels, fibroblasts and hair follicles counts and epithelial thickness were significantly increased in groups II and V as compared with the control group (Table 2).

**DISCUSSION**

Necrosis is a form of cell damage which results in the premature death of cells in living tissues (Nazarian et al., 2009). It is caused by many external factors that effect on the cells or tissues. The standard therapy for necrosis is achieved by removal (debridement) of dead tissues by surgical or non-surgical methods. There are several chemicals for debridement such as fibrinolysin ointment (Lawrence et al., 2012). The effectiveness of papain-urea (mixture of urea and papayas extract, a natural product) was compared to the fibrinolysin ointment and concluded that duration of debridement using papain-urea was shorter (Hebd et al., 1998). Mixture of toasted barley in sesame oil, a traditional herbal medicine, as non-surgical method, for tissue debridement was used in this study. According to the results presented in Figure 1, Tables 1 and 2, in group V, which received 5% w/w dose of toasted barley in sesame oil, the necrotic tissue was reduced, followed by faster wound healing. It seems that the effectiveness of therapeutic dose at 5% w/w toasted barley in the sesame oil for debridement of burn wounds is approximately the same as fibrinolysin ointment.

The classic model of wound healing is divided into three or four sequence, yet overlapping (Anu et al., 2001). Phases: hemostasis (not considered a phase by some authors), inflammation, proliferation and remodeling (Silva et al., 2012). The proliferation phase is characterized by angiogenesis, collagen deposition, tissue granulation, epithelialization, and wound contraction (Midwood et al., 2004). Our results showed that the dose of 5% w/w toasted barley in sesame oil...
Figure 1. Light micrograph of epithelium thickness (arrows) of second-degree burn wounds on 14th (above row) and 28th day (below row). I, control group; II, positive control group and V, group treated with 5% w/v of toasted barley in sesame oil. (H&E staining; ×100).

Figure 2. Healing kinetics of second-degree burn wounds (% wound area): I, control group; II, positive control group and III, IV, V groups were treated respectively with 1, 3, and 5% w/v of toasted barley in sesame oil.

Figure 3. Wound area (%)

Does not only reduce the necrotic tissue, but also improves the wound healing (Figure 3). Chronic inflammation and wound secretions are two sharing factors that cause delay in the burn healing. So, removal of necrotic tissue reduces the inflammation phase and accelerates the wound healing. One of the causes of progressive necrosis is dehydration of the wound due to lack of adequate blood supply (Wang et al., 2010). In this study by considering the amount and type of secretions, no secretions were observed in groups II and V (Table 1). Also, findings indicate that in groups II and V, the color of the wound was more reddish and lacking the necrosis as
compared to the other groups on the 7 and 14th days of study (Figure 3). So, these findings indicate more vascularization in these groups.

The third phase of wound healing is proliferation (Spencer et al., 1996). In this process of wound healing, epithelial cells migrate from the wound edges toward the center, which eventually reduces the wound area. To enable this migration, epidermal cells must lose their connections to the basal membranes and adjacent cells; also expression of keratinocyte receptor is necessary.
formoving to the extracellular matrix. In addition to the keratinocytes, the epidermal derived hair follicles have positive impact on wound healing (Harda et al., 1998). Some of the hair follicles remain healthy in the skin lesions, can produce new epithelial cells and replace the damaged cells during healing process. Consistent with this process, in this study, the hair follicle count was increased in groups II and V (Table 2). Epithelial thickness increasing is the other important factor of wound healing and epithelialization process, leading to growing epithelial islets in the open surface of wounds (Dyer and Roberts, 1990). In another study, the effect of sesame oil was similar to the Bacitracin ointment on the burn wound healing effects (Kogan et al., 2001). In agreement to the aforementioned studies, in groups II and V, the epithelial thickness was increased on the day 14th of this study (Figure 1). Also, the vessels and fibroblasts counts significantly increased in groups II and V on the day 14 (Table 2). The collagen fibers of dermal connective tissue were more regular and dense in group V (Figure 1). It is reported that, ozonized sesame oil has the cutaneous wound healing, increasing wound closure rate and elevation of vascular endothelial growth factors effects (Valacchi et al., 2011). In agreement to the earlier mentioned, as shown in Figures 1 and 2, there is respectively an increase in the epithelial thickness and decrease in the wound area (increasing the wound closure) in groups II and V when compared with the control on days 7, 14, 21 and 28th of the study. These provides insight into the wound healing benefits associated with active material of toasted barley in sesame oil mixture.

Conclusion
The results of this study revealed that the use of toasted barley in sesame oil mixture improved wound healing and tissue debridement. It is suggested that this herbal remedy be used as an alternative non-enzymatic and non-surgical therapy for debridement of burns. Further research is needed to find out the bioactive ingredients especially in combination with other active herbal extracts in order to achieve more effective formulation.

ACKNOWLEDGEMENT
This study was supported by grant (DP/20/18/3/2413, 10/3/2009) from Bushehr University of Medical Sciences, Bushehr, I.R. Iran.

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