Healing potential of Iranian traditional medicinal plants on burn wounds in alloxan-induced diabetic rats

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Abstract: Malva sylvestris, Punica granatum, Amygdalus communis, Arnebia euchroma and Scrophularia deserti are important medicinal plants in Iranian traditional medicine (Unani) whose have been used as remedy against edema, burn, and wound and for their carminative, antimicrobial and anti-inflammatory activities. The ethanol extracts of M. sylvestris and P. granatum flowers, A. communis leaves, A. euchroma roots and S. deserti stems were used to evaluate the burn healing activity in alloxan-induced diabetic rats. Burns were induced in Wistar rats divided into nine groups as following; Group-I: normal rats were treated with simple ointment base (control), Group-II: diabetic rats were treated with simple ointment base (control), Groups-III and –VII: diabetic rats were treated with simple ointment base containing of extracts (diabetic animals), Groups VIII: diabetic rats were treated with simple ointment base containing of mixed extracts, Group-IX: diabetic rats received the standard drug (Silver Sulfadiazine). The efficacy of treatments was evaluated based on wound area, epithelialization time and histopathological characteristics. Wound contraction showed that there is high significant difference between the different groups (p<0.001). At the 18th day, A. euchroma, S. deserti, A. communis and mixed extract ointment treated groups healed 80-90%. At the 9th and 18th days the experiment, the best results were obtained with A. communis and standard drug, when compared to the other groups as well as to the controls. It may be concluded that almond leaves (sweet and bitter) formulated in the simple ointment base is effective in the treatment of burns and thus supports its traditional use.

Keywords: Amygdalus communis Burn wound healing diabetic Iranian traditional medicine

Introduction

Burn injury is a global public health issue especially for the developing and undeveloped countries, which lack adequate medical facilities. Burn injury may lead to complications such as long-term disability, prolonged hospitalization, loss of body extremities and even death. The skin is maintained by a discrete architecture of cells and extracellular matrix which, serves as the principle barrier to environmental and infectious agents. Tissue injuries resulting from burns, frost-bite, gunshots etc. disrupt this barrier, triggering a healing process (Arturson, 1995). Wound healing is a body’s natural process of regenerating dermal and epidermal tissue. The sequence of events that repairs the damage is categorized into three overlapping phases viz. inflammation, proliferation and tissue remodeling (Singer & Clark, 1999). However, burn is characterized by a hypermetabolic state which compromises the immune system leading to chronic wound healing. Thermal exposure to the body surface causes damage to the skin by membrane destabilization, protein coagulation, associated energy depletion and hypoxia at the cellular level which leads to extensive tissue necrosis. Furthermore, the burn wound is a continuous, severe threat against the rest of the body due to invasion of infectious agents, antigen challenge and repeated additional trauma caused by wound cleaning (Arturson, 1995). Healing of wounds, a fundamental response to tissue injury occurs by a process of connective tissue repair. A fibrous scar is the end product of this process, the pre-dominant constituent of which is collagen. Collagen and other components of the ground substance are synthesized by the highly vascular granulation tissue that is formed within the wound space. Collagen provides strength and integrity to the dermis (Raghow, 1994). Infection is a major complication of burn injury and is responsible for 50-75% of hospital deaths (Mokaddas et al., 1998).
Diabetes mellitus is a condition which is known to be associated with a variety of connective tissue abnormalities. The collagen content of the skin is decreased as a result of reduced biosynthesis and/or accelerated degradation of newly synthesized collagen. These abnormalities contribute to the impaired wound healing observed in diabetes (Goodson & Hunt, 1977). Diabetic patients are a special group of patients, known to have an increased risk of wound complications, such as infection and delayed healing. In burn patients, diabetes may have implications for length of hospitalization, hospital course, number of surgical procedures, and burn outcome. A retrospective study was designed in order to identify burn characteristics in diabetic patients admitted to our burn unit, and the impact of diabetes on their hospital course and outcome (Shalom et al., 2005).

Many of the synthetic drugs pose problems such as allergy, drug resistance, etc., forcing scientists to seek alternative drugs (Shannuga Priya et al., 2002). More than 80% of the world’s population depends upon traditional medicines for various skin diseases (Annan & Houghton, 2008). Recently, the traditional use of plants for wound healing has received attention by the scientific community (Annan & Houghton, 2008; Houghton et al., 2005). Approximately one-third of all traditional medicine in use are for the treatment of wounds and skin disorders, compared to only 1-3% of modern drugs (Mantle et al., 2001).

Several plants used as traditional healing remedies have been reported to treat skin disorders, including burn and cut wounds. In Iran, a survey of the ethnomedical studies indicated the use of several of plant species by the inhabitants of the area, especially by those habits the rural areas for burns healing purpose (Ghasemi Pirbalouti, 2009a; Ghasemi Pirbalouti et al., 2009a; Ghorbani, 2003; Zargari, 1989-1992). For example, tribal (Chaharmahal va Bakhtiar) in South West Iran, roots of Arnebia euchroma (Ghasemi Pirbalouti et al., 2009a), stems of Scrophularia deserti (Ghasemi Pirbalouti et al., 2010a) and leaves of sweet and bitter almond (Amygdalus communis) used as remedy burn wound (Ghasemi Pirbalouti, 2009b).

Many traditional remedies are based on systematic observations and methodologies and have been time-tested but for many of them, scientific evidence is lacking. There are only few prospective randomized controlled trials that have proved the clinical efficacy of these traditional burns healing agents. The present study was designed to test the in vivo burn wound healing activity of the ethanol extracts of five selected medicinal plants, namely; Malva sylvestris L. (Malvaceae), Punica granatum L. (Punicaceae), Amygdalus communis L. (Rosaceae), Arnebia euchroma Rolye. (Johnst) (Boraginaceae) and Scrophularia deserti Del. (Scrophulariaceae).

### Material and Methods

#### Plant material

The Malva sylvestris, Punica granatum, Amygdalus communis and Arnebia euchroma were collected from mountain areas of Zagross, Chaharmahal va Bakhtiar, South-West Iran, and Scrophularia deserti was collected from Ilam, West Iran, during May-June, 2009. Provisional identifications of specimens were made with the help of “Flora of Iran” (Ghahreman, 1987–1989), “Flora of Ilam” (Mozaffarian, 2008), “Encyclopedia of Iranian Plants” (Mozaffarian, 1996), “Flora Iranica” (Rechinger, 1963-1998) etc. In addition Mr Shirmardi and Mr Prirani, Research Centre of Agriculture and Natural Resources, Ministry of Agriculture, Iran, authenticated the plants.

#### Extract preparation

About 100 g of powdered flowers of M. sylvestris and P. granatum, leaves of A. communis (sweet and bitter), roots of A. euchroma and stems of S. deserti were extracted with absolute ethanol (Merck, Germany) using Soxhlet apparatus for 12 h. The concentrated extract were filtered using Whatman No. 1 filter paper and then lyophilized gave a green residue with yield 5.8% for M. sylvestris, a red residue with 10.5 % w/w for P. granatum, a dark red residue with 12 % w/w for A. euchroma and green residue with 8.9 % and 6.5 % w/w for A. communis and S. deserti, respectively. The extract samples were stored in universal bottles and refrigerated at 4 °C prior to use.

#### Experimental animals

Male Wister rats (150-180 g) of two months were used. The animals were housed in standard environmental conditions of temperature (22±3 °C), humidity (60±5%) and a 12-h light/dark cycle. During experimental time Wistar rats were given standard pellet diet (Pastor Institute, Iran) and water ad libitum. The rats were used for the experiment after one week of acclimatization period. All the procedures were approved by the Medical Ethics Committee of Shahrekord University of Medical Sciences.

#### Diabetic animals

After 15 h fasting, rats were intraperitoneally treated daily with 125 mg/kg alloxan monohydrate (Sigma chemicals, St Louis, USA) freshly dissolved in distilled water (5%) for two consecutive days (Diatewa et al., 2004). Blood was drawn from the orbital plexus 24 h after the injection and the glucose level was estimated.
Burns were made on the rats showing elevated blood glucose (>250 mg/dL).

**Burn induction**

Animals were anesthetized with 1.5 mg/kg i.p. of Ketamin and Xylazine and their dorsal surface was shaved with a sterile blade. The shaved area was disinfected with 70% (v/v) ethanol. The burn wounds were created using the method described by Shanmuga Priya et al. (2002) with some modifications. A cylindrical metal rod (15 mm diameter) was heated to 80-90 ºC and pressed to the shaved and disinfected surface for 20 s in rat under Ketamin and Xylazine anesthesia. The animals were randomly divided into nine groups each containing six animals.

**Grouping of animals**

Burns were induced in Wistar rats divided into nine groups as following; Group-I: normal rats were treated with simple ointment base, Group-II: diabetic rats were treated with simple ointment base (control), Groups-III and -VII: diabetic rats were treated with simple ointment base containing of extracts (diabetic animals), Groups VIII: diabetic rats were treated with simple ointment base containing of mixed extracts (1:1), Group-IX: diabetic rats received the standard drug (Silver Sulfadiazine-Najo 1%) at 200 mg/kg/day dose in all groups.

**Measurement of wound area**

The progressive changes in wound area were measured in cm² by tracing the wound boundaries on a transparent paper on every 3-day interval. The burn wound area was calculated using Auto CAD RL 14 software.

**Evaluation of histopathology**

At the 9th and 18th days the experiment was terminated and the wound area was removed from the surviving animals for histological examination. The excision skin biopsies were fixed in 10% formaldehyde solution 48 h during the experimentation period and were embedded in paraffin wax. A 6 µm thickness sections were stained with hematoxylin–eosin stain and observed for the histopathological changes under light microscope (Olympus BX51). Inflammatory cell (neutrophil), re-epithelisation, angiogenesis, fibroblasts, vascularisation, Extracellular matrix, vascularization and organization of the collagen were qualitatively evaluated by grading as (-), (+), (++), (+++).

**Evaluation of microbiological status**

The microbiological status of the wounds was determined by taking sterile swabs of each burn on the 9th and 18th days. These swabs were streaked onto sterile nutrient agar plates and incubated for 24 h at 37 ºC. Colony counts were recorded and Gram stains performed on representative colonies.

**Analysis of data**

Results were expressed as mean±SEM. The differences between experimental groups were compared using one-way Analysis of Variance (ANOVA) and significant means were separated using Duncan’s multiple range test (DMRT). Differences were considered significant at p<0.001. All data processing was performed with SPSS software Version 11.5.

**Results and Discussion**

**Wound contraction**

Wound area was traced manually and was photographed in each three days interval and healed area calculated by subtracting from the original wound area. The percentage wound contraction was determined using the following formula:

\[
\text{Percent wound contraction} = \frac{\text{Healed area}}{\text{Total wound area}} \times 100
\]

To apply this equation, the wound margins were traced and measured to calculate the non-healed area which was then subtracted from the original wound area to obtain the healed area. Wound contraction on different days is shown in Table 1. Statistically, the percentage of wound contraction showed that there is high significant difference between the different groups (p<0.001). The wound healing potential for *A. euchroma*, *S. deserti*, *A. communis* and mixed extracts was evident on the 18th day (Table 1). No healing effect was observed with *P. granatum* (Table 1).

**Epithelialization time**

The Epithelialization time was found be high significantly (p<0.001) reduced in groups as depicted in Table 2. A better healing pattern with complete wound closure was observed in treated within 21 days while it was about 34 days in non-diabetic control rats. At the 18th day, *A. euchroma*, *S. deserti*, *A. communis* and mixed extract ointment treated groups healed 80-90% and standard drug group showed 51% healing (Table 2).
Alloxan-induced diabetic rats

Each value represents mean±SEM; -no detected.

At the 9th and 18th days the experiment, evaluation of microbiological status was carried out for groups. At 9th and 18th days, lowest colonies per swab of Bacillus and Staphylococcus species were detected in group of mixed extracts (Table 5), highest colonies were detected in groups of diabetic control and P. granatum extract.

Despite the traditional uses M. sylvestris and P. granatum, A. communis (sweet and bitter), A. euchroma and S. deserti in burn wound healing process in Iran, there are no reported data available in the literature. These species widely distributed plants of Iran are used for the anti-infectious, anti-inflammatory, antimicrobial, skin disease and for wound and burn healing properties according to several ethnobotanical surveys (Ghasemi Pirbalouti, 2009a; Ghasemi Pirbalouti et al., 2009; Ghasemi Pirbalouti et al., 2010b, Ghorbani, 2003; Zargari, 1989-1992). The present study tested the burn wound-healing properties of the ethanol extracts of M. sylvestris, P. granatum, A. communis, A. euchroma, S. deserti and mixed extracts were used to evaluate the burn healing activity in alloxan-induced diabetic rats.

On the different days, the results of morphological evaluation showed that A. communis, A. euchroma, S. deserti and mixed extracts significantly increased the percentage of wound contraction (Table 1). At the 18th day the experiment, A. communis extract and standard drug (Silver Sulfadiazine) showed increased collagen turnover (Tables 2-4). Collagen, the major component which strengthens and supports extracellular tissue, is composed of the amino acid, hydroxyproline, which has been used as a biochemical marker for tissue collagen (Philips et al., 1991).

Wound healing is a process by which damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of the area of the wound (Nayak et al., 2007). It is mainly composed of the amino acid, hydroxyproline, which has been used as a biochemical marker for tissue collagen (Philips et al., 1991).

**Histological evaluation**

At the 9th and 18th days the experiment, histological evaluation was carried out for the treated and untreated samples. Comparison between controls and some treated animals is shown in Tables 3 and 4. The best results were obtained with A. communis and standard drug, when compared to the other groups as well as to the controls. On the 18th day, groups of A. communis extract and standard drug showed complete healing as in collagenation, fibroblasts cells and angiogenesis in Table 4. The control groups (diabetic and non-diabetic rats) and some of the groups (P. granatum) presented edema, monocyte cells and area with cellular necrosis (Table 4).

**Evaluation of microbiological status**

At the 9th and 18th days the experiment, microbiological evaluation was carried out for groups. At the 9th and 18th days, lowest colonies per swab of Bacillus and Staphylococcus species were detected in group of mixed extracts (Table 5), highest colonies were detected in groups of diabetic control and P. granatum extract.
dependent upon the type and extent of damage, the general state of health and the ability of the tissue to repair. The aims in these processes are to regenerate and reconstruct the disrupted anatomical continuity and functional status of the skin (Philips et al., 1991). Wound contracture is a process that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. In the maturation phase, the final phase of wound healing, the wound

### Table 3. Effect of the treatments on the evolution of wounds in rats after nine days of topical application.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Inflammation cells (Neutrophil)</th>
<th>Angiogenesis</th>
<th>Re-epithelization</th>
<th>Fibroblasts</th>
<th>Extracellular matrix</th>
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<tr>
<td>Simple ointment</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>++</td>
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<tr>
<td>(non diabetic)</td>
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<tr>
<td>Silver Sulfadiazine</td>
<td>+</td>
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<td>(diabetic rats)</td>
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<tr>
<td>Punica granatum</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
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<tr>
<td>(diabetic rats)</td>
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<tr>
<td>Malva sylvestris</td>
<td>+</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Scrophularia deserti</td>
<td>+</td>
<td>++</td>
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<td>+</td>
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<tr>
<td>(diabetic rats)</td>
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<tr>
<td>Amygdalus communis</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
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<tr>
<td>(diabetic rats)</td>
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</tr>
<tr>
<td>Mixed extracts</td>
<td>+</td>
<td>+++</td>
<td>+/-.</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+: slight, ++: moderate, +++: extensive, -: absent.

### Table 4. Effect of the treatments on the evolution of wounds in rats after eighteen days of topical application.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Inflammation cells (Neutrophil)</th>
<th>Collagen maturation</th>
<th>Re-epithelization</th>
<th>Organization of the collagen</th>
<th>Vascularization</th>
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<tr>
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<tr>
<td>Simple ointment</td>
<td>+++</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Silver Sulfadiazine</td>
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<td>++</td>
<td>+/+.</td>
<td>+++</td>
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<tr>
<td>(diabetic rats)</td>
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<tr>
<td>Punica granatum</td>
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<td>++</td>
<td>+</td>
<td>+++</td>
<td>++</td>
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<tr>
<td>(diabetic rats)</td>
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<tr>
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<td>-</td>
<td>++</td>
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<td>+</td>
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<td>(diabetic rats)</td>
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<tr>
<td>Amygdalus communis</td>
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<td>+++</td>
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</tbody>
</table>

*: slight, ++: moderate, +++: extensive, -: absent.
undergoes contraction resulting in a smaller amount of apparent scar tissue. Granulation tissue formed in the final part of the proliferative phase is primarily composed of fibroblasts, collagen, edema, and new small blood vessels. The increase in dry granulation tissue weight in the test treated animals suggests higher protein content (Philips et al., 1991).

The results of study showed that the extract ointment of *M. sylvestris*, *P. granatum*, *A. communis*, *S. deserti* and mixed extracts effectively stimulates burn wound contraction as compared to controls group. These finding could justify the inclusion of these plants in the management of wound healing. The result of the present study offers pharmacological evidence on the folkloric uses of *M. sylvestris* flowers, *P. granatum* flowers, *A. communis* leaves and *S. deserti* stems for healing burn wound. Hence, the results support the traditional uses of *M. sylvestris*, *P. granatum*, *A. communis* and *S. deserti* to treat skin disorders including burns.

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