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Effect of Natural Remedies on Dead Space Wound Healing in Wistar Rats

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ABSTRACT

The natural remedies include the chloroform extract of *Malva sylvestris* Linn flowers (4%), the ethanol extract of *Punica granatum* Linn flowers (2%), aqueous extract of *Achillea kellalensis* Boiss flowers (2%), and honey (2%) were used to evaluate the wound-healing activity on male Wistar rats (150–200 g) using excision and incision wound models. A 1.0×1.5-cm full thickness excision wound was made in the dorsal area of the rats. The animals were divided randomly into three groups of nine each. Group 1 was treated with nitrofurazone ointment and served as a reference standard (200 mg/kg/day); Group 2 was treated topically with the simple ointment prepared from natural remedies (200 mg/kg/day); Group 3 (control) was treated topically with the simple ointment (200 mg/kg/day). The treated animals by natural remedies showed reduction in the wound area when compared with nitrofurazone and control. Histological studies of the tissue obtained on days 6, 9, and 16 from natural remedies showed increased well-organized bands of collagen, more fibroblasts, and a few inflammatory cells when compared with the nitrofurazone and control which showed inflammatory cells, scanty collagen fibers, and fibroblasts. Enhanced wound contraction and histological findings suggest the use of natural remedies in the management of wound-healing.

Keywords: natural remedies; excision; wound; medicinal plants.

INTRODUCTION

Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following the injury, an inflammatory response occurs and the cells below the dermis (the deepest skin layer) begin to increase collagen (connective tissue) production. Later, the epithelial tissue (the outer skin layer) is regenerated (9). Wound care can be treated back to early civilizations, and many of these treatments were based on the use of herbal remedies. Approximately, one-third of all traditional medicines in use are for the treatment of wounds and skin disorders, compared to only 1–3% of modern drugs (8).

A survey of the ethnobotanical studies, carried out in Iran, indicated the use of several of plant species by the inhabitants of the area, especially by those habiting the rural areas for wound-healing purpose (3–15). The common ways of treatment are either by direct application of crushed fresh/dried plant on the wound or by repeated wash of the wound with a plant decoction. Despite the widespread use of Iranian herbs in wound-healing, only few of them has been investigated for their potential usefulness using excision and incision wound models by rats (5).

Reports about medicinal plants affecting various phases of the wound-healing process, such as coagulation, inflammation, fibroplasia, collagenation, epithelization, and wound contraction are abundant in the scientific literature (1–9).

Malva sylvestris Linn (Malvaceae), known locally as "Panirak", is a medicinal plant in Iran whose flowers are

used for the treatment of various ailments, including cold, cough and burn, and cut wound-healing in rural areas of Iran (15). *Achillea kellalensis* (Asteraceae), a well-known traditional herb used in tribal medicine of Iran is locally known as "Kallari Bumadaran or Golberenjas". The decoction of the leaves and flowers is being used by the tribal people of Chaharmahal and Bakhtyari for the treatment of skin infection, ulcer, and antibacterial infections (3). *Punica granatum* Linn, known locally as "Golnar-e-farsi", is an important medicinal plant in Iran whose flowers are used an astringent, hemostatic, antimicrobial and as a remedy for cut wound, bronchitis, diarrhea, digestive problems, dermal infected wounds, and diabetes in *Unani* medicinal (Iranian Traditional Medicine) literatures (3–15).

No systematic studies has yet been carried out on the clinical evaluation of the wound-healing potency of natural remedies (complex extracts: *M. sylvestris, P. granatum A. kellalensis*, and honey), so its effects were investigated using excision, incision, and dead space wound-repair models in rats.

MATERIALS AND METHODS

Plant material and extract preparation

The flowers of *M. sylvestris, P. granatum*, and *A. kellalensis* were collected from mountain areas of Zagross, district of Chaharmahal va Bakhtiari, Iran, during May–June, 2007. Their identity was confirmed and voucher specimens were deposited at the researches center of medicinal plant and ethno-veterinary (RCMPEV), Islamic Azad University, Iran. Powdered flowers of *M. sylvestris* (100 g) were Soxhlet-extracted with 80% chloroform and ethanol (Mark, Germany) for 2 h (yields: 6% and 10 %), respectively. The flower powder (100 g) of *A. kellalensis* was refluxed with 350 ml of double distilled water for 2 h at 70°C. The infusions were filtered and concentrated under rotary vacuum (model Zirbus 302®) for about 1 h (yield: 5.5%).

Experimental animals

Male Wister rats (150–200 g) of 2–3 months were used as experimental animals. 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*.

Wound-healing activity

Wound induction and evaluation extracts for properties wound healing before the beginning of the wound-healing experiments, the dorsal skin of the Wistar rats were shaved. Animals were anesthetized with 1.5 ml/kg *i.p.* of Ketamin and Xylazine. A full thickness of the excision wound (circular area about 150 mm^2 and 2-mm depth) was created along the markings using toothed forceps, a surgical blade, and pointed scissors. The animals were divided randomly into three groups of nine each. Group 1 was treated with nitrofurazone ointment and served as a reference standard (200 mg/kg/day); Group 2 was treated topically with the simple ointment prepared complex of *M. sylvestris, P. granatum, A. kellalensis* extracts and honey (200 mg/kg/day); Group 3 (control) was treated topically with the simple ointment (200 mg/kg/day).

The percentage of wound closure was calculated as follows by using the initial and final area drawn on glass slides during the experiments (13):

% of wound closure = (Wound area on day 0 × Wound area on day *n*)/Wound area on day 0 × 100

*n*_ number of days (4th, 8th, 12th, and 16th day).

On days 6th, 9th, and 16th, the experiment was terminated and the wound area was removed from the surviving animals for histological examination. The excision skin biopsies were fixed in 4% formaldehyde solution for 48 h during the experimentation period.

Analysis of data

The relative wound area was statistically analyzed as mean ± SD and statistically significance between treated and control groups were analyzed by means of student's *t*-test. Data are significant, $p \leq 0.05$ compared with control.

RESULTS

Morphological evaluation

During the wound-healing period and at present time intervals, the wound area was traced manually and photographed. The wound area was calculated using AutoCAD RL 14 software. The animals treated with the natural remedies showed a significant reduction in the wound area when compared with nitrofurazone and control (Table 1). They also showed faster epithelialization than those treated with the positive control. The extract-treated animals by natural remedies showed 99% reduction in the wound area when compared with control.

Histological evaluation

The study of the histological structure showed that the tissue regeneration was greater in the skin wound treated with the ointment containing the complex extracts of *M. sylvestris, P. granatum, A. kellalensis*, and honey (Table 2). The

Table 1: Effect of the treatments on wound healing in rats

Treatments	Wound area relative (cm^2)		
	6 days	9 days	16 days
Malva sylvestris + *Punica granatum* + *Achillea kellalensis* + honey	0.729±0.14**	0.157±0.032***	0.051±0.014**
Nitrofurazone	0.77±0.060**	0.33±0.043**	0.077±0.047*
Control (simple ointment)	1.379±0.075	1.133±0.070	0.226±0.040

Each value represents mean ±S.D. *N= 9 animals.*
Statistically significant by Student's *t*-test.
***P≤0.01,*
**P ≤ 0.05* levels of significance.

Table 2: Effect of the treatments on the evolution of wounds in rats after 6, 9 and 16 days of topical application

Treatments	Inflammation cells			Collagen fibers			Re-epithelization			Organization of the collagen			Necrosis			Fibrin		
	6 th	9 th	16 th	6 th	9 th	16 th	6 th	9 th	16 th	6 th	9 th	16 th	6 th	9 th	16 th	6 th	9 th	16 th
Malva sylvestris + *Punica granatum* + *Achillea kellalensis* + honey	+	–	–	+	++	+++	+	+	++	++	++	+++	–	–	–	+	+	–
Control Simple ointment	+++	++	+	–	–	+	–	–	+	–	–	+	+++	++	+	+++	++	+
Standard drug Nitrofurazone	+	+	–	+	+	++	+	+	++	+	++	++	+	–	–	++	+	–

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

skin wound treated with simple ointment and presented edema, monocyte cells and area with cellular necrosis that were not observed in the treated with ointment containing the natural remedies (Table 2).

CONCLUSION

Wound-healing is a process by which a damaged tissue is restored as closely as possible to its normal state whereas wound contraction is the process of shrinkage of the area of the wound (10). It is mainly dependent upon the type and extent of damage, the general state of health, and the ability of the tissue to repair. Despite the Iranian traditional uses complex extracts of *M. sylvestris, P. granatum, A. kellalensis*, and honey in wound-healing process in Iran, there are no reported data available in the literature. *M. sylvestris, P. granatum*, genus of *Achillea*widely distributed plants of Iran are used for the infectious, antiinflammatory, antimicrobial, skin disease, and for wound-healing properties according to several ethnobotanical surveys (3–15).

In present study, results of wound area measurements, as shown in Tables 1 and 2, indicated a healing potential for the complex of *M. sylvestris, P. granatum, A. kellalensis* extracts, and honey. Statistically, area measurements showed that there is significance among the different groups. The results of histological evaluation showed that natural remedies have increased the rate of wound contraction and collagen turnover. 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 (6).

The preliminary phytochemical analysis of the flower extract by researchers showed the absence of anthocyanin, malvin, malvidin 3-(6"-malonylglucoside)-5-glucoside, malvaline, niacin, and folic acid. Any one of the phytochemical constituents (malvone A: 2-methyl-3-methoxy-5, 6-dihydroxy-1,4-naphthoquinone) present in *M. sylvestris* may be responsible for antimicrobial activity and antiinflammatory activities (2–4). Monoterpenoids include camphor, borneol, *a*-thujone, cineol, bornyl acetate, and camphene present in *A. kellalensis* flowers may be responsible for antimicrobial activity and antiulcer (11). Polyphenol compound named pomegranatate present in *P. granatum* includes ellagic acid, 3,3',4'-tri-*O*-methylellagic acid, ethyl brevifolincarboxylate, urolic, maslinic acids, and daucosterol may be responsible for antiinfected of dermal (12–14). Finally, these may be either due to the individual or additive effect of the phytoconstituents that hasten the process of wound-healing. Further, phytochemical studies are needed to isolate the active compound(s) responsible for these pharmacological activities.

In conclusion, while plants and honey-based traditional medicine have been used throughout generations, the efficiency of such treatments requires experimental backup and scientific verification. In this study, plant species presented were selected based on ethnopharmacological information, provided by local communities. However,

the enhanced wound contraction and histological observations suggest that the complex extracts of *M. sylvestris, P. granatum, A. kellalensis*, and honey have potential in the management of wound healing and requires further study.

REFERENCES

1. Asif A., Kakub G., Mehmood S., Khunum R. and Gulfraz M. Wound healing activity of root extracts of *Berberis lyceum* Royle in rats. *Phytother. Res* **21** (2007), pp. 589–591.
2. D'Amelio F.S. 1999. *Botanicals, a Phyto-cosmetic Desk Reference*, CRC Press LLC, p. 361.
3. Ghasemi A., Pirbalouti A and Golparvar A.R. 2007. *Evaluation of ethnobotany in the region of Chaharmahal va Bakhtyari, West Central Iran. Proceedings of the international symposium on medicinal and nutraceutical plants*: Macon, Georgia, USA, March 19-23, 2007. Acta horticulture (ISSN 0567-7 572) p. 756.
4. Ghorbani A. Studies on pharmaceutical ethnobotany in the region of Turkman Sahra, north of Iran. *J Ethnopharmacol* **102** (2005), pp. 58–68.
5. Hemmati A.A. and Mohammadian F. An investigation into the effects of mucilage of quince seeds on wound healing in rabbit. *J Herbs Spices and Medicinal plants* **7**, (2000), pp. 41–46.
6. Khalil E.A., Afif F.U. and Al-Hussainin M. Evaluation of the wound healing effect of some Jordanian traditional medicinal plants formulated in pluronic F127 using mice (*Mus musculus*). *J Ethnopharmacol* **109** (2006), pp. 104–112.
7. Lusby P.E., Coombes A.L. and Wilkinson J.M. A comparison of wound healing following treatment with *Lavandula* x *allardii* honey or essential oil. *Phytother Res* **21** (2006), pp. 755–757.
8. Mantle D., Gok M.A. and Lennard T.W.J. Adverse and beneficial effects of plant extracts on skin and skin discorders. *Adverse Drug Reaction and Toxicological Reviews* **20** (2001), pp. 89–103.
9. Nayak B.S., Godwin I., Davis E.M. and Pillai G.K. The evidence based wound healing activity of *Lawsonia inermis* Linn. *Phytother Res* **21** (2007), pp. 827–831.
10. Senthil Kumar M., Kirubanandan S., Sripriya R. and Kumar Sehgal P. Triphala promotes healing of infected full-thickness dermal wound. *J Surgical Res* **144** (2006), pp. 94–101.
11. Rustaiyan A., Masoudi S. and Yari M. The essential oils of *Achillea aucheri* Boiss. and *A. kellalensis* Boiss. et Hausskn. from Iran. *J Essent Oil Res* **11** (1999), pp 19–20.
12. Tom H. W., Huang G.P. and Bhavani P.K. Anti-diabetic action of *Punica granatum* flower extract: Activation of PPAR-γ and identification of an active component *Toxicol Appl Pharmacol* **207** (2005), pp.160–169.
13. Wall S. J., Bevan D. and Thomas D.W. Differential expression of matrix metalloproteinases during impaired wound healing of the diabetes mouse. *J Invest Dermatol* **119** (2002), pp. 91–98.
14. Wang R., Wang W., Wang L., Liu R., Ding Y. and Du L. Constituents of the flowers of *Punica granatum*. *Fitoterapia* **77** (2006), pp. 534–537.
15. Zargari A. 1989–1992. *Medicinal Plants*. University Publication, Tehran (in Persian).