

The Healing Potential of Oleaster Leave Water Soluble Extract on Experimental Skin Wounds in the Rat

Mohammad Mehdi Oloumi^{*1} DVSc
Amin Derakhshanfar² PhD
Mohammad Mehdi Molaei¹ DVSc
Parisa Falahi³ DVM

¹Department of Clinical Studies and ²Department of Pathobiology,
Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman,
Kerman, Iran.

³Graduated from the Faculty of Veterinary Medicine,
Shahid Bahonar University of Kerman, Kerman, Iran.

Abstract

Objective- To evaluate the healing potential of oleaster leave water soluble extract on skin wounds.

Design- Experimental study.

Animals- 30 male Spragne-Dawly rats, randomly assigned in two equal groups.

Procedures- Under general anesthesia, and following surgical preparation, two uniform skin defects were made by 7mm skin punch. The water soluble extract was then applied on half of the wounds for 7 days. The animals were sacrificed on day 8 and histopathologica and biomechanical samples were taken. Wound planimetry was also performed.

Results- All the evaluated parameters in histopathological and biomechanical studies were significantly superior in treatment group in comparison with the control one. The surface area of the treatment wounds were also significantly less than the control wounds.

Conclusion and Clinical Relevance- This study showed that oleaster leave extract has a pro-healing effect on the experimental skin wounds and it can be considered as an available herbal preparation with reasonable cost.

Key Words- Oleaster leave extract, wound healing, histopathology, biomechanics, rat.

*Corresponding author:

Mohammad Mehdi Oloumi DVSc

Department of Clinical Studies, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman,
Kerman, Iran.

E-mail address: oloumi@mail.uk.ac.ir

Introduction

Wound repair is a symphony of biological processes, initiated by tissue injury that culminates in restoration of tissue integrity. It involves reformation of barriers to fluid loss and infection besides limiting further entry to foreign organisms and material. Eventually, wound healing re-establishes normal blood and lymphatic flow patterns, and restores mechanical integrity of the injured system. Normal wound healing follows a predictable pattern that can be divided into overlapping phases defined by cellular populations and biochemical activities: (a) hemostasis and inflammation, (b) proliferation and (c) maturation and remodeling¹.

The use of plants as medicines goes back to early man. Certainly the great civilization of the ancient Chinese, Indians, Iranians and North Africans provided written evidence of man's ingenuity in utilizing plants for the treatment of wide variety of diseases².

In Iran, the number of pharmacies is proportional to the number of inhabitants, at least in the cities. But Iran is a vast country, where many villages are far from the cities, in these villages there are only a few pharmacies. For this reason, folk herbal medicine is the most used remedy to cure common and widespread diseases³, including wounds and burns.

There are some herbal remedies mentioned in ancient Iranian medicinal texts, believed to be effective in treating wounds.

Oleaster (Russian olive) (Senjed, in Persian), is a tree from the family *Elaeagnaceae*, genus *Elaeagnus*. It has oval, arrow-shaped leaves with small yellow flowers which are very aromatic. Its olive-like fleshy fruit has thin skin and sweet taste. In ancient Iranian medicinal texts, its fruit has been administered for cough, as a diuretic, antiemetic and astringent. The powdered dried leaves of the tree were also applied directly on wounds to control hemorrhage and accelerate wound healing⁴.

In this study the wound healing potential of water soluble extract of oleaster leaves (OLE) has been examined on experimental wounds in rats.

Materials and Methods

Preparation of OLE

Powdered air-dried leaves of oleaster tree was soaked in 80°C distilled water (1:3 weight:volume) for 40 min. The mixture was then passed through paper filter and the clear liquid put in 50°C water bath until a honey like consistency with 20% moisture achieved. The sterile solution was kept in 4°C refrigerator⁵.

The animal used

30 male Spragne-Dawley rats, weighing 180-200 grams were randomly assigned to two equal groups (treatment and control). The animals were housed under controlled condition (12 h. light-dark cycle, 22° C, 60% humidity). They were fed a rodent chow and had tap water *ad lib*. All the procedures were conducted in accordance with the European community guidelines for laboratory animals.

Wound creation and treatment schedule

The back of animals in the thoraco-lumbar region was surgically prepared for aseptic surgery. A circular wound was created on each side by a 7-mm biopsy punch (total of 90 wounds). The

surgical procedures were carried out under general anesthesia (90 mg/kg ketamine + 10 mg/kg xylazine).

All the wounds (treatment and control) were rinsed daily by 10 ml sterile normal saline. In treatment wounds (30 wounds) following rinsing, the OLE was applied topically on each wound by sterile cotton tip soabs once daily for 7 days, whereas the control wounds (30 wounds) were remained untreated. Wounds remained uncovered in both groups throughout the experiment.

Wound harvesting and evaluations

After 7 days the animals were sacrificed by intracardiac injection of 20 mg/kg thiopental sodium. For histopathological studies (15 wounds from each group), regenerated tissues were cut in the form of square pieces along with normal skin on either side of the wound and preserved in 10% buffered formalin. Following routine preparation of tissues, serial sections of paraffin embedded tissues of 5 μm thickness were cut with a microtome and stained with hemotoxylin and eosin and studied under light microscope for fibroblastic proliferation, angiogenesis and re-epithelialization. A new method was used to quantify the histopathological data. For this purpose, the depth of granulation tissue in each slide was measured by objective micrometry lens under 40 \times magnification. A field at the middle of this length was then considered and the number of fibroblasts and capillary buds were counted under 100 \times and 400 \times magnifications, respectively and presented in number per mm^2 . To evaluate re-epithelialization, the epithelial gap was measured under 200 \times magnification. The values were averaged for each group⁵.

For biomechanical studies (15 wounds from each group) a strip of skin, 7 cm. long, with the same widths of wound diameter, in the manner that the wound was located at the middle of the strip, was removed by a double-blade scalpel. The skin was then wrapped in Ringer's soaked gauze, aluminum foils and plastic bags and kept in -20 $^{\circ}$ C freezer until tensile testing. The samples were defrosted by immersing in 20 $^{\circ}$ C Ringer's solution. The samples were then mounted in a Strograph mechanical test frame (Toyoseiky Tensile Testing Unit, model R3, Japan) fitted with appropriate clamps, the distance between the clamps at the start of testing being 4 cm. The strips were loaded with 0-50 kg load cell, with strain rate of 1cm/min. and the load-elongation curves were drawn. The following parameters were measured from the load-elongation curves: yield strength (yield point) (kg), ultimate strength (kg), maximum stored energy (kg.cm), and stiffness (kg.cm).

Measurement of wound area

Wound area was measured before wound excision in order to determine unhealed wound area (raw wound) by drawing wound boundaries around it on transparent paper and the area within the boundary was calculated by using graph paper. The values for each treatment were averaged and presented in mm^2 .

Statistical analysis

Student's t-test was used to compare two means. A value of $P \leq 0.05$ was considered as significant.

Results

Wound area

As shown in fig. 1, the wound area in treatment group was significantly less than the control group (21.17 ± 3.14 and 30.25 ± 3.75 , respectively).

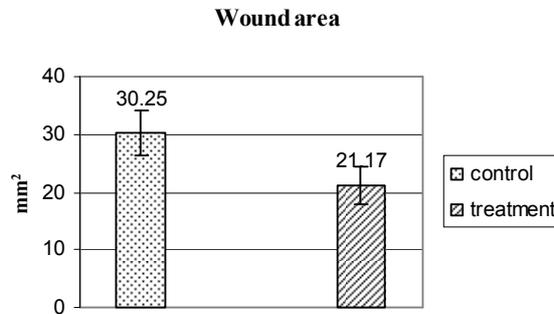


Fig. 1. The surface area of the wounds in two groups.

Histopathological parameters

The number of capillary buds (1015.25 ± 146.45) and fibroblasts (1318.33 ± 126.18) in treatment group were significantly more than the control group (601.03 ± 51.32 and 701.98 ± 98.65 , respectively). The epithelial gap in treatment group was also significantly less than the control group (131.67 ± 22.09 and 1031.13 ± 98.69 , respectively) (fig. 2-6).

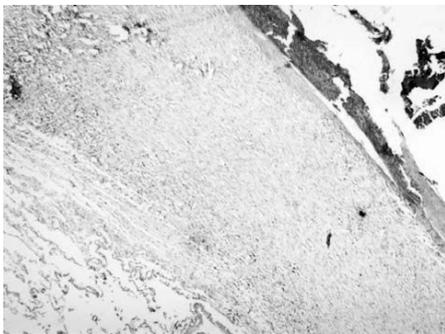


Fig. 2: Complete re-epithelialization in treatment group (40×, H&E)

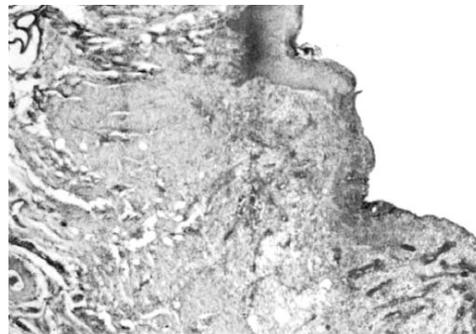


Fig. 3: Incomplete re-epithelialization in control group (40×, H&E)

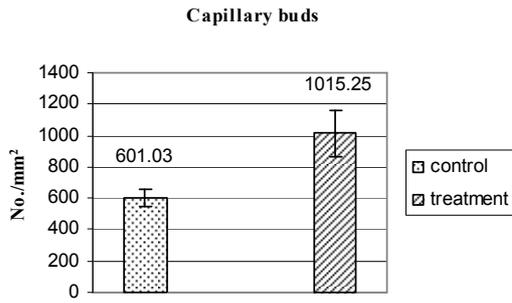


Fig. 4: The number of capillary buds observed in the histopathological samples.

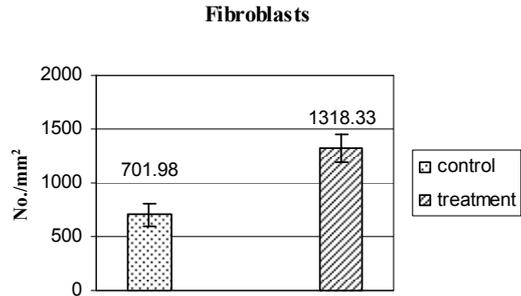
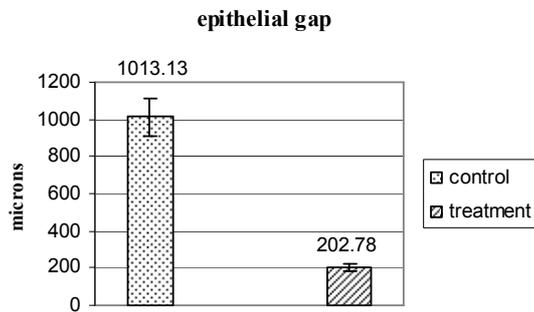


Fig. 5: The number of fibroblasts observed in the histopathological samples.

Fig. 6: The epithelial gap, measured in histopathological samples.



Biomechanical parameters

The yield point, ultimate strength, stiffness and maximum stored energy in treatment group were 1.75 ± 0.26 kg, 1.25 ± 0.19 kg, 0.98 ± 0.27 kg.cm and 1.44 ± 0.27 kg.cm, respectively. These amounts for control group were 0.69 ± 0.21 , 0.81 ± 0.18 , 0.51 ± 0.20 and 0.72 ± 0.15 , respectively. The differences between the two groups in all the biomechanical parameters were statistically significant (fig. 7-10).

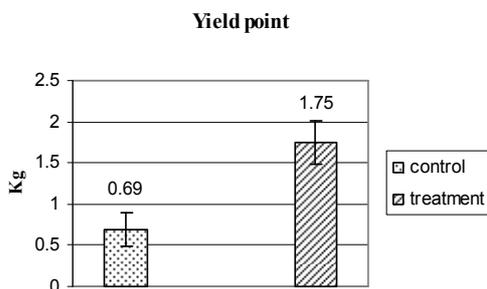


Fig. 7: The yield point of the samples calculated from load-elongation curves.

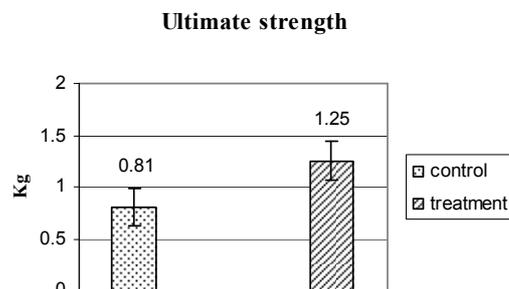


Fig. 8: The ultimate strength of the samples, calculated from load-elongation curves.

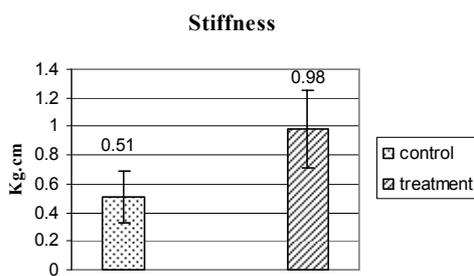


Fig.9: Stiffness of the samples, calculated from load-elongation curves.

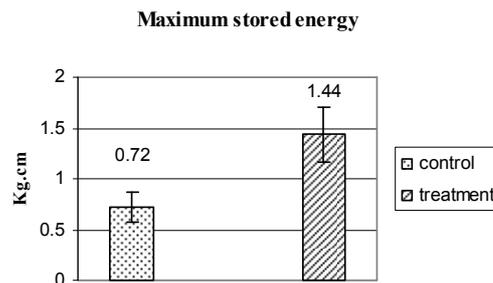


Fig.10: Maximum stored energy of the samples, calculated from load-elongation curves.

Discussion

The treatment of skin loss due to severe and massive burns or wounds continues to be a major problem in surgical procedures. A therapeutic agent selected for the treatment of wounds should ideally improve one or more phases of healing without producing deleterious side effects⁶. Skin wound healing, which starts immediately after injury, consists of three phases: inflammation, proliferation, and maturation. These phases proceed with complicated but well-organized interaction between various tissues and cells^{7,8}. Angiogenesis is indispensable for sustaining granulation tissue⁹. As shown in fig.1, the number of capillary buds and fibroblasts in treatment group were significantly more than the control one. It shows that the rate of angiogenesis was higher under the influence of the plant extract. Also application of OLE at the wound caused significant wound healing activity. Increased fibroblastic proliferation may be due to mitogenic activity of the extract, which might have significantly contributed to healing process. Fibroblasts are the most common cells in skin connective tissue and are the adhering cells which are thought to play a customary role in wound healing assistance¹⁰. Early dermal and epidermal regeneration, as shown by reduced epithelial gap and wound surface area (figs.1 and 4) and massive angiogenesis (fig. 2) in treated rats also confirmed that the extract had a positive effect towards cellular proliferation, granulation tissue formation and re-epithelialization¹¹.

Figs. 5-8 show that, all the biomechanical parameters in treatment group are significantly higher than the control. Skin contains collagen fibers, which are arranged in a criss-crossed pattern and characterize the mechanical properties of the tissue¹²⁻¹⁴. In this study the biomechanical results are consistent with the histopathological results. Increased fibroblastic proliferation results in increased collagen synthesis¹⁵. Collagens are the main extracellular component of the skin. During the proliferative phase of skin wound healing, the synthesis of different proteins of particularly collagen subtypes within wounds increases to replace necrotic tissue^{9,10}. Collagen not only confers strength and integrity to the tissue matrix, but also plays an important role in homeostasis and epithelialization at the later phase of healing^{12,16}. Therefore, enhanced synthesis of collagen provides strength to repaired tissue and also healing pattern¹⁵.

Considering the findings of this study, it can be concluded that the local administration of a water soluble extract of oleaster leave could improve the different phases of wound healing, including collagen synthesis and maturation, wound contraction and epithelialization.

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References

1. Kaushal M, Kutty NG, Rao CM. Wound healing activity of NOE-aspirin: A pre-clinical study. *Nitric Oxide* 2007;16:150-156.
2. Philipson JD. Phytochemistry and medicinal plants. *Phytochemistry* 2001;56:237-243.
3. Miraldi E, Ferri S, Mostaghimi V. Botanical drugs and preparations in the traditional medicine of West Azarbaijan (Iran). *J Ethnopharmacol* 2001;75:77-87.
4. Zargari A. *Medicinal Plants*. 4th ed. Vol. 2. Tehran: Pouyesh Pub Co, 1992;770-772.
5. Derakhshanfar A, Oloumi MM, Kabootari J, et al. Histopathological and biomechanical study on the effect of *Artemisia sieberi* extract on experimental skin wound healing in rat. *Iran J Vet Surg* 2006;1:36-42.
6. Sumitra M, Mnikandan P, Suguna L. Efficacy of *Butea monosperma* on dermal wound healing in rats. *IJBCB* 2005;37:566-573.
7. Singer AJ, Clark RA. Cutaneous wound healing. *N Engl J Med* 1999;341:738-746.
8. Martin P. Wound healing – aiming for perfect skin regeneration. *Science* 1997;276:75-81.
9. Kondo T. Timing of skin wounds. *Legal Med* 2007; in press, doi:10.16/j.legalmed.2006.11.009
10. Lu WW, Ip WY, Jing WM, et al. Biomechanical properties of thin skin flap after basic fibroblast growth factor (bFGF) administration. *Br J Plast Surg* 2000;53:225-229.
11. Shanmuga Priya K, Gnanamani A, Radhakrishnan N, et al. Healing potential of *Datura alba* on burn wounds in albino rats. *J Ethnopharmacol* 2002;83:193-199.
12. Lees VC, Fan TP. A freeze-injured skin graft model for the quantitative study of basic fibroblast growth factor and other promoters of angiogenesis in wound healing. *Br J Plast Surg* 1994;47:349-359.
13. Gibson T, Kenedi RM. Biomechanical properties of skin. *Surg Clin North Am* 1967;47:279-294.
14. Irie T, Oka H, Yamamoto T. Measurement of hardness of human skin with impact force. *Med Biol Eng Comput* 1994; 32: 231-233.
15. Nayak BS, Udupu AL, Udupa SL. Effect of *Ixora coccinea* flowers on dead space wound healing in rats. *Fitoterapia* 1999;70:233-236.
16. Clark RAF. 1996. Wound repair. Overview and general considerations. In: R.A. Clark RA, Henson PM, eds. *The Molecular and Cellular biology of Wound Repair.*, New York: Plenum Press, 1996:3-27.

بررسی اثرات التیامی عصاره آبی برگ گیاه سنجد بر زخم های تجربی پوست در موش صحرایی

دکتر محمد مهدی علومی^۱، دکتر امین درخشانی^۲، دکتر محمد مهدی مولایی^۱، دکتر پریسا فلاحی^۲

^۱ گروه علوم درمانگاهی و ^۲ گروه پاتوبیولوژی دانشکده دامپزشکی،
دانشگاه شهید باهنر کرمان، کرمان، ایران.

^۲ دانش آموخته دانشکده دامپزشکی دانشگاه شهید باهنر کرمان، کرمان، ایران.

هدف: بررسی اثرات التیامی عصاره آبی برگ سنجد بر زخم های تجربی پوست در موش صحرایی.
طرح: مطالعه تجربی.

حیوانات: ۳۰ رأس موش صحرایی اسپراگن-دولی که بطور تصادفی در دو گروه قرار گرفتند.

روش کار: تحت بیهوش عمومی و متعاقب آماده سازی جراحی، دو نقیصه پوست متحدالشکل با استفاده از پانچ ۷ میلی متری در طرفین پشت حیوانات ایجاد شد. عصاره آبی برگ گیاه سنجد بر نیمی از زخم ها به مدت ۷ روز قرار گرفت. حیوانات در روز ۸ قربانی شدند و نمونه گیری برای بررسی های هیستوپاتولوژیک و بیومکانیک از پوست انجام گرفت. اندازه گیری سطح زخم نیز انجام شد.
نتایج: کلیه پارامترهای مورد بررسی هیستوپاتولوژیک و بیومکانیک در گروه درمان بطور معنی داری بالاتر از گروه شاهد بود. سطح زخم نیز در گروه درمان در پایان دوره بطور معنی داری کمتر از گروه شاهد بود.

نتیجه گیری: این مطالعه نشان داد که عصاره آبی برگ گیاه سنجد دارای تأثیرات التیامی بر زخم تجربی پوست موش صحرایی می باشد و می توان از آن به عنوان روشی ارزان قیمت در درمان زخم ها استفاده نمود.

کلید واژگان: عصاره برگ گیاه سنجد، التیام زخم، هیستوپاتولوژی، بیومکانیک، موش صحرایی.