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Original Study

Histopathological and Biomechanical Study on the Effect of *Artemisia sieberi* Extract on Experimental Skin Wound Healing in Rat

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Abstract

Objective- To evaluate the effects of *Artemisia sieberi* extract on experimental dermal wounds healing.
Design- Experimental study.
Animals- 30 male Spragne-Dawly rats.

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Procedures- Two uniform 7 mm diameter skin defect were created on the back of each animal by skin punch (total of 60 wounds). Then, the extract of the plant was applied once daily on half of the wounds for histopathological and biomechanical examinations.

Results- *Artemisia sieberi* extract caused a significant increase in the number of fibroblasts and capillary buds and also significant decrease in the epithelial gap which showed the better formation of healing tissue in treatment group. On the other hand,the improvement of biomechanical indices in treatment group revealed a significant increase in tensile strength of the wounds.

Conclusion and Clinical Relevance- It can be concluded that *Artemisia sieberi* is an effective herbal remedy in wound healing.

Key Words- *Artemisia sieberi,* Skin, Wound healing

Introduction

Nowadays, we observe the strong return of herbal preparations to medical sciences. Herbal treatments can often be traced back thousands of years, and plants are the source of many of today·s successful prescription medicines.[1] Although, Iran is one of the main foci for the medicinal plants growing, many ethnobotanical papers have been published in literature about Africa, India and south America, but very few papers have been written concerning the medicinal use of plants in Iran.[2] Artemisia is a world wide medicinal plant finds in Europe, America, Asia and especially in vast areas of Iran such as Mahabad, Kashan, Ardestan, Yazd, Sabsevar, Damghan, Tehran, Varamin and Kerman.[3] Artemisia has been used for more than 2000 of years in Chinese traditional medicine as antipyretic, antimalaria, disinfectant, analgesic, antiulcerogenic compound and antidiarrhea. This plant consists of various chemical substances such as alkaloids, flavonoids, tannins and artemisin as the major component. Previous studies showed the effect of this component on Plasmodium in drug resistant malaria and also it·s anticoccidial effects.[4] In a more recent report the larvicidal action of Artemisia extract against malaria vector(Anopheles) is demonstrated.[5] For the first time, the effect of *Artemisia sieberi* on experimental skin wound healing is investigated by the present study.

Materials and Methods

Prepration of the extract: Aerial parts of the *Artemisia sieberi* were collected from the area of Yazd province and transferred to the laboratory. After washing and drying in 28 ° C temperature, they were crushed and extracted in the Soxhlet apparatus with petrolium benzene for the 6 h in 30 – 50° C temperature. Solvents were removed by vacume rotary evaporator to obtain extract. 4 ml of ethanol was added to extract and put on shaker for 5 min. Subsequently the ethanol extract was filtered and kept in 4° C refrigerator .[3]

Experimental design and sampling: 30 male Spragne-Dawley rats, weighing 180-200 grams 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. 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 60 wounds). The surgical procedures were carried out under general anesthesia (ketamine, 90 mg/kg IM + Xylazine, 10 mg/kg IM).

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

After 10 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. 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° C freezer until tensile testing.

Histopathological examination: Following routine preparation of tissues, serial sections of paraffin embedded tissues of 5 ¼m 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× magnification. A field at the middle of this length was then considered and the number of fibroblasts and capillary buds were counted under 100× and 400× magnifications, respectively and presented in number per mm^2. To evaluate re-epithelialization, the epithelial gap was measured under 200× magnification (fig. 1). The values were averaged for each group.

Biomechanical evaluation: The samples were defrosted by immersing in 20° 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).

Statistical analysis: Student's t-test was used to compare two means. A value of Pd"0.05 was considered as significant.

Results

Histopathology: The number of fibroblasts and capillary buds were significantly (Pd"0.05) higher in treatment group (Table 1). Also the epithelial gap in treatment group (fig. 2) was significantly less than the control group (fig. 3), showing better re-epithelialization in this group.

Biomechanic: All biomechanical parameters measured in this study were significantly higher in treatment group compared with control group (table 2), which shows better biomechanical properties of the treated tissues.

Table 1: Histopathological parameters, evaluated in treatment and control groups.
* significant difference between the two groups (P≤0.05)

	Histopathological parameters *		
	Cappillary buds (No./mm^2)	Fibroblast (No./mm^2)	Epithelial gap (μ)
Treatment	1121.55 ± 146.28	1171.67± 125.27	143.71 ± 20.83
Control	399.12 ± 32.85	666.91 ± 93.21	944.47 ± 64.52

Table 2: Biomechanical parameters, evaluated in treatment and control groups.
Y.P: yield point, U.S: ultimate strength, Stif.: stiffness, M.E.S: maximum stored energy. * significant difference between the two groups (P≤0.05)

	Biomechanical parameters *			
	Y.P (Kg)	U.S (Kg)	Stif.(Kg.cm)	M.E.S(Kg.cm)
Treatment	1.11 ± 0.18	1.21 ± 0.24	0.88 ± 0.17	1.16 ± 0.26
Control	0.63 ± 0.14	0.71 ± 0.13	0.42 ± 0.15	0.61 ± 0.18

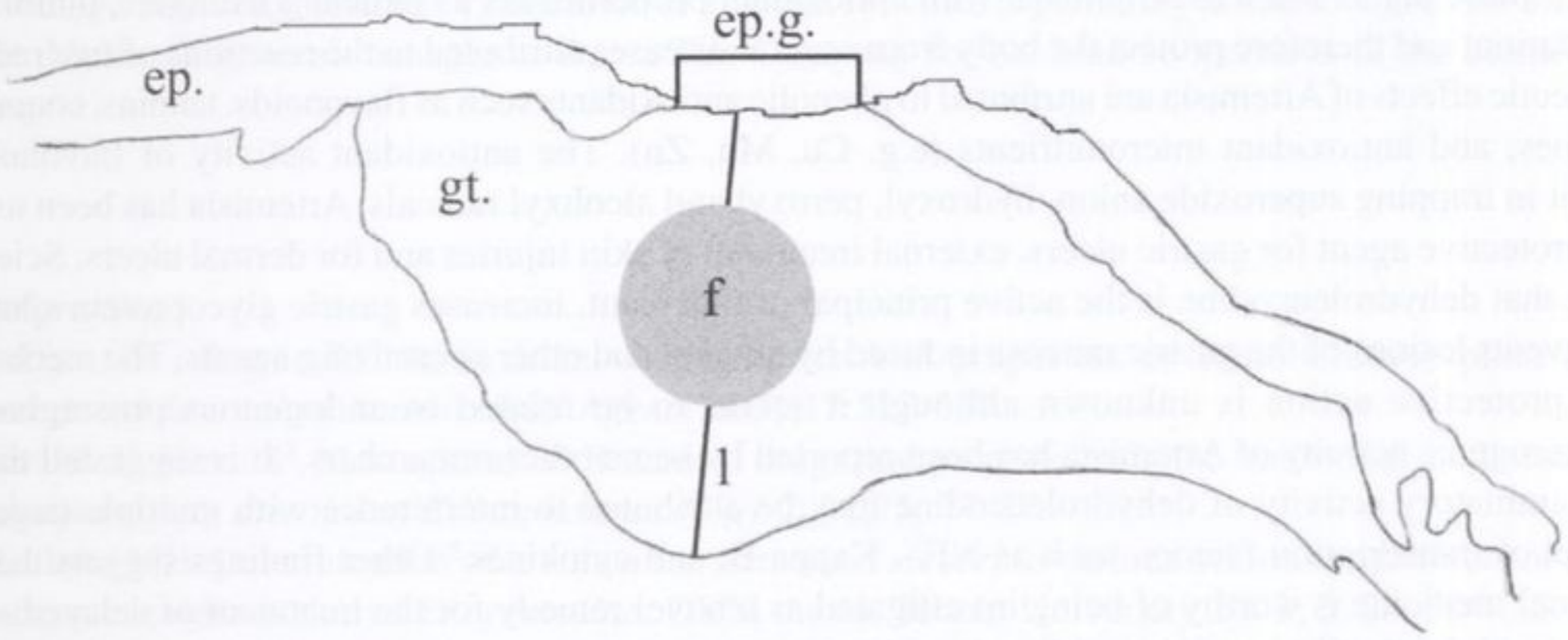

Fig. 1: Schematic drawing showing the new method used for quantitative tissue characterization. ep: epithelium, ep.g: epithelial gap, f: the field in which the number of fibroblasts and capillary buds were counted. g.t: granulation tissue, l: the depth of granulation tissue.

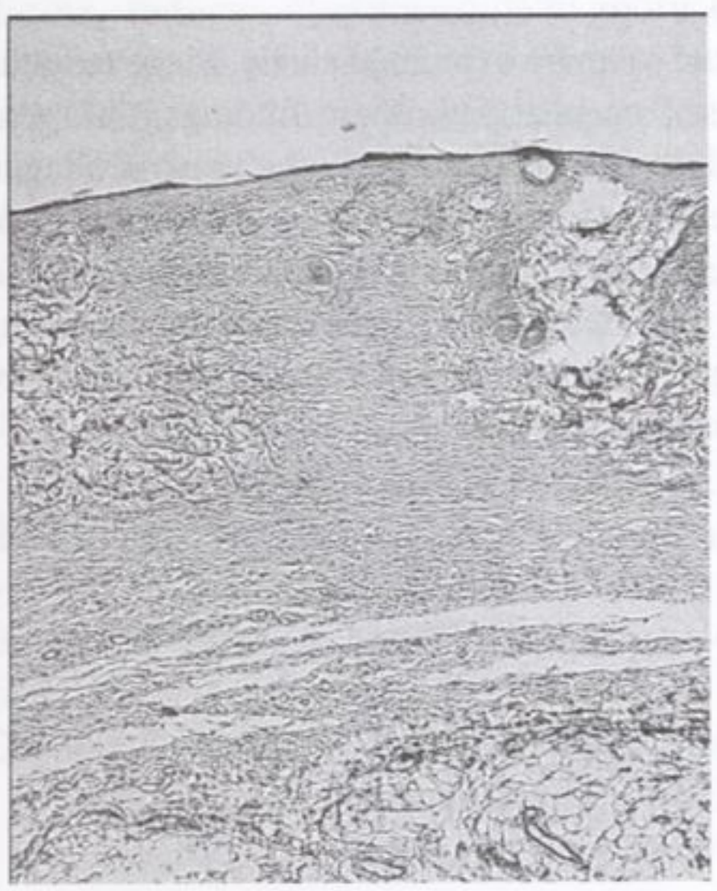

Fig. 2: The epithelial gap in treatment group (H&E, 40×).

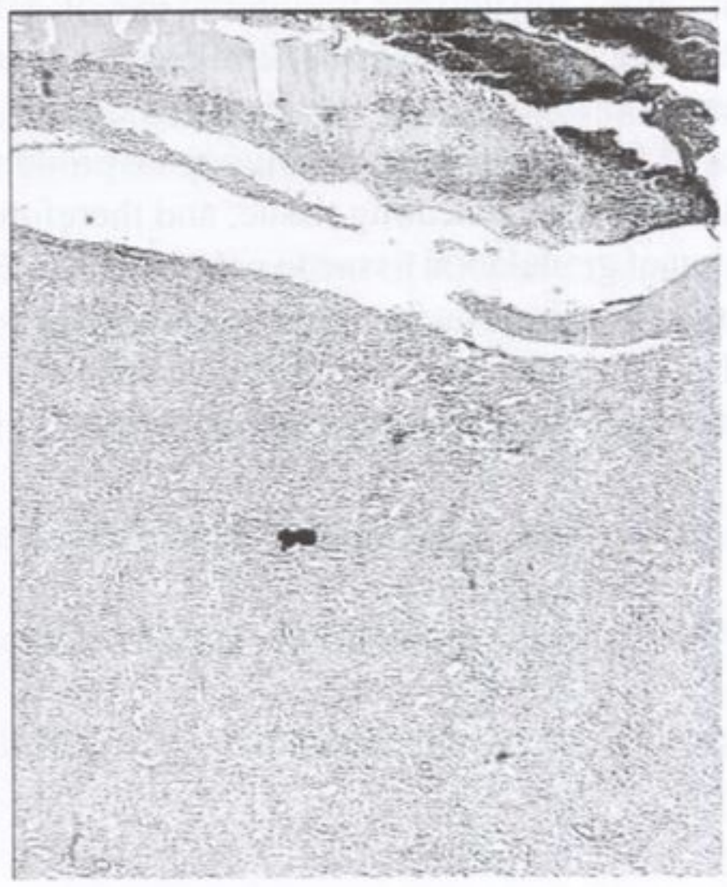

Fig. 3: The epithelial gap in control group (H&E, 40×). Compare the gap with the figure 2.

Discussion

The origins of the therapeutic use of herbal medicine can be traced back to China about 5000 years ago. Many of these plants ,such as Artemisia, with antioxidant properties act as radical scavengers, inhibit lipid peroxidation, and therefore protect the body from several diseases attributed to the reactions of free radicals. Therapeutic effects of Artemisia are attributed to phenolic antioxidants such as flavonoids, tannins, coumarins, xanthenes, and antioxidant micronutrients (e.g. Cu, Mn, Zn). The antioxidant activity of flavonoids is efficient in trapping superoxide anion, hydroxyl, peroxyl and alcohxyl radicals. Artemisia has been used as a cytoprotective agent for gastric ulcers, external treatment of skin injuries and for dermal ulcers. Scientists believe that dehydroleucodine is the active principal of this plant, increases gastric glycoprotein synthesis and prevents lesions of the gastric mucosa induced by ethanol and other necrotizing agents. The mechanism of the protective action is unknown although it seems to be related to endogenous prostaglandin.[7] Antiulcerogenic activity of Artemisia has been reported by some other researchers.[8] It is suggested that the antiinflammatory activity of dehydroleucodine may be attributed to interference with multiple targets on the level of transcription factors, such as NF – Kappa B, and cytokines.[9] Other findings suggest that this traditional medicine is worthy of being investigated as a novel remedy for the treatment of delayed – type hyper sensitivity, inhibition of lymphocyte proliferation and reduction in IL – 2 production.[10] Scoparone is a major component of the shoot of Artemisia which has been used for the treatment of hepatitis and biliary tract infection in oriental countries. Scoparone decreases the production of the inflammatory mediators such as nitric oxide and prostaglandin E_2 in macrophages by inhibiting inducible nitric oxide synthase and cyclooxygenase – 2 expression.[11] Several in vitro studies have demonstrated that flavonoids affecting various steps in the arachidonate cascade via cyclooxygenase – 2 or lipoxygenase resulted in decrease of inflammation.[7] It was suggested that Artemisia extract stimulated endothelial cell proliferation by increasing the production of basic fibroblast growth factor, and therefore the more production of healing tissue.[12] The antimicrobial activities of four species of Artemisia of Iran are reported.[13] Animal experiment has demonstrated that qinghao acid is one of the actively bacteriostatic constituents of the Artemisia.[14] The hepatoprotective effect of this plant was verified by histopathology of the liver, which showed improved architecture, absence of paranchyma congestion, decreased cellular swelling and apoptic cells. These findings scientifically validated the traditional use of Artemisia for various liver disorders.[15]

According to aforementioned reasons the results of this study can be explained easily. Since reduction of inflammation predisposes the injured tissue to healing, based on histopathologic findings, the increased number of fibroblasts is due to mitogenic properties of the plant, results in more production of collagen and more strength of the healing tissue, and therefore re – epithelialization. Increase in capillary buds results in formation of granulation tissue in a shorter time. Biomechanical results confirm the histopathological findings obviously. The improvement of biomechanical indices, in treatment group, is indebted to increasing the fibroblasts and collagen and also decreasing of epithelial gap.[16] In conclusion, *Artemisia sieberi* extract accelerates the healing process in skin wound via various mechanisms.

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چکیده:

مطالعه هیستوپاتولوژیک و بیومکانیک اثر عصاره گیاه درمنه سیبری بر التیام زخم های تجربی پوست در موش صحرائی

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هدف: بررسی اثر عصاره گیاه درمنه سیبری ژ در التیام زخم های تجربی پوست در رت
طرح: مطالعه تجربی
حیوانات: ۳۰ سر موش صحرائی نر از نژاد اسپراگ-دولی
روش کار: و به کمک پانچ بیوپسی دو زخم یکسان (مجموعاً ۶۰ زخم) به قطر ۷ میلی متر در دو طرف ستون مهره ها در پشت هر حیوان ایجاد شد. آنگاه عصاره گیاه درمنه سیبری به مدت ۱۰ روز-روزی یکبار- بروی نیمی از زخم ها قرار داده شد. در پایان آزمایش حیوانات بطریق انسانی کشته شدند و نمونه های لازم جهت مطالعات هیستوپاتولوژی و بیومکانیک اخذ گردید
نتایج: از افزایش معنی دار فیبروبلاست ها و جوانه های مویرگی و نیز کاهش معنی دار فاصله دو انتهای بافت پوششی در حال تشکیل در گروه آزمایش بود که خود بیانگر شکل گیری موثر تر بافت التیامی در این گروه است. از سوی دیگر بهبود شاخص های بیومکانیک در گروه آزمایش حکایت از افزایش استحکام کششی زخم ها داشت
نتیجه گیری: عصاره گیاه درمنه سیبری در التیام زخم های پوستی موثرمی باشد.
کلید واژه ها: درمنه سیبری، التیام زخم پوست