Healing potential of liquorice root extract on dermal wounds in rats

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Abstract: Liquorice root extract (LE) has been used from long ago as an effective medication, especially in gastric ulcer. In this study, the healing potential of aqueous LE on dermal wounds was evaluated. The study was carried out on 45 male Sprague-Dawley rats. Two uniform 7mm-diameter skin defects were created on the back of each animal by 7mm skin punch (total of 90 wounds). LE was applied once daily on half of the wounds for 7 days, after which the animals were sacrificed for histopathological, biochemical (hydroxyproline content) and biomechanical studies. The ultimate surface area of the wounds was also measured. LE caused a significant increase in the number of fibroblasts and capillary buds, collagen contents and tensile strength of the wounds. The wound surface area in the treatment group was also significantly less than the control group. It can be concluded that LE is an effective herbal remedy in wound healing.

Key words: liquorice root extract, skin wound healing.

Introduction

Liquorice root extract (LE), derived from the plant Glycyrrhiza glabra has long been used in medicine. It is used as a flavoring and sweetening agent for American-type tobaccos, chewing gums, candies, etc. (Dehpour et al., 1995; Fuhrman et al., 2002; Nomura et al., 2002; Kitagava, 2002; Paolini et al., 1998). Clinical studies have shown that LE has spasmolytic and beneficial influence on healing process of gastric ulcer (Bennet et al., 1985; Tylor et al., 1988). Also, antiarthritic (Paolini et al., 1998), antiallergic (Kumagai et al., 1967), antiviral (Pompe et al., 1979), antitumor, antifibrotic (Kiso et al., 1984), anticholinergic, antiestrogenic, anti-inflammatory (Paolini et al., 1998), antileukaemic (Logemann and Lauria, 1960), anticarcinogenic (Mirsalis et al., 1993) and antiatherosclerotic (Fuhrman et al., 2002) actions are only some of the possible therapeutic properties of LE and one of its constituents, glycyrrhizin (Paolini et al., 1998, 1999).

On the other hand, wound healing involves a series of well coordinated biochemical and cellular events leading to the growth and regeneration of wounded tissue in a specific manner. Participation of various inflammatory cells such as macrophages and neutrophils is extremely crucial to the repair process. These cells also promote the migration and proliferation of endothelial cells, leading to neovascularization of connective tissue cells, which synthesize the extracellular matrices including collagen; and of keratinocytes leading to re-epithelialization of the wounded tissue (Clark, 1991; Rasik et al., 1999). These intervening events are controlled by the coordinated action of certain specific growth factors and cytokines by acting on target cells at the site of injury (Bennet and Schultz,
Unfortunately, all these processes involved in tissue repair are altered during pathological conditions such as diabetes, immune disorders and ischemia and in injuries such as burns and gunshot wounds. In such conditions, wound does not heal perfectly. It would be interesting to unravel the possible mechanisms involved in such cases as this would help to identify precisely newer agents, which may improve the delayed healing process (Rasik et al., 1999).

In an ancient Iranian medical text published between 1895 and 1898 (Dagha’egh Al’alaj; "details of treatment", written by Haj Mohammad Karim Kermani), powdered liquorice root was administered directly on the wounds and believed that it could exaggerate wound healing. Considering this old remedy and the ethnopharmacological profile and reputed medicinal use in traditional practice, the present study was undertaken for the first time to evaluate the possible dermal wound healing potential of aqueous LE.

Materials and Methods

Preparation of LE: Powdered roots of Glycyrhriza glabra were 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 was achieved. The sterile solution was kept in refrigerator at 4°C.

Animal: 45 male Sprague-Dawley rats, weighing 180-200 grams were housed under controlled conditions (12 h. light-dark cycle, 22°C, 60% humidity). They were fed 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: 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).

Treatment Schedule: All the wounds (treatment and control) were rinsed daily by 10 ml sterile saline solution. In the treatment group (45 wounds) following rinsing, LE was applied topically by sterile cotton tip soaks once daily for seven days, whereas the control wounds (45 wounds) were remained untreated. Wounds remained uncovered in both groups throughout the experiment.

Wound Harvesting: After seven days, the animals were sacrificed by intracardiac injection of 20 mg/kg thiopental sodium. For biochemical analysis, 15 wounds from each group were excised by the same punch used for wound creation and care was taken to chop off only the newly formed regenerated tissue without any contamination with normal skin (Rasik et al., 1999). The samples were kept at -70°C for biochemical analysis. 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.

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 mm².

Collagen Content: Hydroxyproline, the basic constituent of collagen was taken as a marker of collagen synthesis. Seven days treated tissue was dried in hot air oven at 60-70°C till consistent weight was achieved. These dried samples were hydrolyzed with 6N HCl for 4 h at 130°C. The hydrolyzed samples were adjusted to pH 7 and subjected to chloramines T oxidation and finally the colored adduct formed with Ehrlich reagent at 60°C was read at 557 nm after cooling for 5 min. Standard hydroxyproline was also run concurrently and values
Table 1: Wound area and hydroxyproline content of treatment and control group.

<table>
<thead>
<tr>
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<th>Wound area * (mm²)</th>
<th>hydroxyproline content * (mg/g dry wt. Tissue)</th>
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<tbody>
<tr>
<td>Treatment</td>
<td>18.28 ± 3.70</td>
<td>83.14 ± 10.15</td>
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<tr>
<td>Control</td>
<td>28.12 ± 3.25</td>
<td>49.35 ± 8.65</td>
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* significant difference between the two groups (p<0.05).

Table 2: Histopathological parameters, evaluated in treatment and control groups.

<table>
<thead>
<tr>
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<th>Histopathological parameters *</th>
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<tbody>
<tr>
<td></td>
<td>Capillary buds (No./mm²)</td>
</tr>
<tr>
<td></td>
<td>Fibroblast (No./mm²)</td>
</tr>
<tr>
<td></td>
<td>Epithelial gap (µ)</td>
</tr>
<tr>
<td>Treatment</td>
<td>1158.66 ± 157.30</td>
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<td></td>
<td>1119.81 ± 116.21</td>
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<tr>
<td></td>
<td>150.75 ± 21.92</td>
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<tr>
<td>Control</td>
<td>402.13 ± 34.87</td>
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<td></td>
<td>674.93 ± 94.29</td>
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<td></td>
<td>950.48 ± 65.53</td>
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* significant difference between the two groups (p<0.05).

Table 3: Biomechanical parameters, evaluated in treatment and control groups.

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<th>Biomechanical parameters *</th>
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<tr>
<td></td>
<td>Y.P (Kg)</td>
</tr>
<tr>
<td></td>
<td>U.S (Kg)</td>
</tr>
<tr>
<td></td>
<td>Stif. (Kg.cm)</td>
</tr>
<tr>
<td></td>
<td>M.E.S (Kg.cm)</td>
</tr>
<tr>
<td>Treatment</td>
<td>1.13 ± 0.21</td>
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<tr>
<td></td>
<td>1.18 ± 0.22</td>
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<tr>
<td></td>
<td>0.90 ± 0.20</td>
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<tr>
<td></td>
<td>1.23 ± 0.31</td>
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<tr>
<td>Control</td>
<td>0.65 ± 0.16</td>
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<td></td>
<td>0.72 ± 0.16</td>
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<td></td>
<td>0.44 ± 0.16</td>
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<tr>
<td></td>
<td>0.64 ± 0.19</td>
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* significant difference between the two groups (p<0.05).

reported as mg/g dry wt. of tissue.

**Histopathological Study:** 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 eosiin 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 the 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 mm². To evaluate the re-epithelialization, the epithelial gap was measured under 200× magnification (fig. 1). The values were averaged for each group.

**Biomechanical Study:** The samples were defrosted by immersing in 20°C Ringer’s solution. The samples were then mounted on a Strograph mechanical test frame (Toyoseiki Tensile Testing Unit, model R3, Japan) fitted with appropriate clamps, with the distance between the clamps at the start of the testing being 4 cm. The strips were loaded with 0-50 kg load cell, with a strain rate of 1 cm/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 p<0.05 was considered as significant.

**Results**

**Wound Area:** The area of wound in the treatment group was 35% less than the control group (Table 1).

**Hydroxyproline Content:** Hydroxyproline content in the treatment group was significantly higher than the control group (p<0.05), indicating enhanced synthesis of collagen (table 1).

**Histopathological Results:** As shown in Table 2, the number of fibroblasts and capillary buds was significantly higher in the treatment group (p<0.05). Also the epithelial gap in the treatment group (fig. 2) was significantly less than the control group (fig. 3), showing better re-epithelialization in this group.

**Biomechanical Results:** All biomechanical parameters measured in this study were significantly higher in the treatment group compared with the control group (table 3), which shows better biomechanical properties of the treated tissues.
Discussion

Wound healing is a complex process characterized by hemostasis, re-epithelialization, granulation tissue formation and remodeling of the extracellular matrix. Though, healing process takes place by itself and does not require much help, but various risk factors such as infection and delay in
healing have been brought to attention to promote this process (Shanmuga Priya et al., 2002).

Topical application of LE on 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. Early dermal and epidermal regeneration and massive angiogenesis in treated rats also confirmed that the extract had a
positive effect towards cellular proliferation, granulation tissue formation and re-epithelialization (Shanmuga Priya et al., 2002).

Biochemical analysis showed increased hydroxyproline content, which is a reflection of increased fibroblastic proliferation and thereby increased collagen synthesis (Nayak et al., 1999). 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 (Clark, 1996). Collagen is one of the most dominant extracellular matrix proteins in the granulation tissue, which appears to be significantly high by the fifth day of wounding and after day seven, collagen production is further advanced (Grillo, 1964). Therefore, enhanced synthesis of collagen provides strength to repaired tissue and also healing pattern (Shanmuga Priya et al., 2002).

Our biomechanical results well correlate with biochemical and histopathological results. Gain in tensile strength correlates with the rate of collagen synthesis through the first 10 weeks of healing (Capperauld, 1989; Freeman et al., 1989; Madden and Peacock, 1971; Miro et al., 1995; Paul et al., 1997). As shown in Table 1, maximum load and stiffness of the treated tissues were significantly higher than the control one. Maximum load, which is the functionally most important parameter for characterizing healing wounds (Quirinia and Vidiik, 1991), reflects the ultimate tensile strength of the specimen, at which complete failure occurs rapidly, and load supporting ability of the tissue is substantially reduced (Carlstedt and Nordin, 1989). This happens as the intermolecular cross links are broken and the collagen fibrils pass each other or as collagen fibrils lose contact with ground substance (Freeman et al., 1989). Higher stiffness and maximum load of the treated tissue confirm our biochemical and histopathological observations regarding more fibroblasts, less epithelial gaps and increased collagen synthesis in the treatment group.

Size reduction in treatment wounds was also significantly more than the control ones, which can be attributed to prominent re-epithelialization in treated wounds, which was confirmed microscopically by measuring epithelial gap.

We can conclude that the remedy prescribed by Kermani in 1890s in the book "Daghalegh Afala" must have been effective on wound healing, although leaving the particles of non-sterile powdered root in an open wound must have had some negative effect on the healing process.

Considering the results of the present study, it can also be concluded that LE possesses some pro-healing activity, which can affect the process of wound healing at various phases of tissue repair.

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References


بررسی خواص الکیومی عصاره ریشه گیاه شیرین بیان بر زخم‌های پوستی در موش صحرایی

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پذیرش نهایی: ۱۳۹۸/۰۹/۱۱

عمل کردن شیرین بیان در زمان‌های دور به عنوان یک داروی مؤثر بر خیمی و مولکولی بیماری‌های درمان و درمان مجدد مورد استفاده قرار گرفته است. در این مطالعه، اثر الکیومی عمل کردن در زخم‌های صحرایی موش در روش‌های تولید قرار گرفته، به این منظور از ۴۵ موش صحرایی سفید نژاد نراده امریکی استفاده شد. این موش‌ها در نهایت به عنوان گروه اصلی انتخاب گردیدند. برای این منظور، نتایج مطالعات قبلی همبستگی ویژه‌سازی (نمونه‌برداری کربن یا عصاره) همبستگی میکروبی و ویژگی‌های مکانیکی نتایج بهتری تهیه کنند. نتایج نهایی در زخم‌های بهپایه زده‌ها و استحکام در چسب زخم‌های درمان‌شده کشف شد.

از نظر کلی، عمل کردن شیرین بیان در زخم‌های صحرایی موش باعث رشد‌زا و درمان مجدد زخم‌های صحرایی موش می‌شود.