

Effect of Licorice on Wound Healing In Rabbits

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Abstract

Licorice (*Glycyrrhiza glabra*) is one of the most widely used medicinal plants and is included in numerous traditional and modern medical preparations. In the present study the healing effect of licorice extract was investigated on open skin wounds in rabbits. Adult Newzeland rabbits of both sexes weighing 1.5 - 2.5 kg were used. Hairs of lower back and left flank of animal were shaved. Full-thickness wound (15x15 mm) was made on the shaved area. Hydroalcoholic extract of licorice was prepared by maceration method. Creams of 5%, 10% and 15% (w/w) extract in eucerin base were prepared and applied 2 times daily. Dexpanthenol ointment was used as standard control. Healing was determined by reduction in wound area. The results of this study proved that licorice cream of 10% was a potent healing agent even better than dexpanthenol cream.

Introduction

Healing of wounds or restoration of tissues integrity is a natural mechanism and normal reaction to injury. Inflammation, proliferation and remodeling, all are the main steps of wound healing. Wound healing in animals and humans is relatively similar (Champion *et al.*, 1998). Many drugs of chemical or natural origin as dexpanthenol, Ginko biloba, phenytoin, zinc oxide, and others have been used topically to improve the rate of wound healing (Lawrence *et al.*, 1995; Hemmati & Mohammadian, 2000; Bairy & Rao, 2000).

Licorice (*Glycyrrhiza glabra*) is a plant that grows wild in Western and Eastern world and has long history of therapeutic uses by cultures throughout the world, including the Greeks, Egyptians, Chinese and Indians. It was traditionally used in treatment of peptic ulcer, asthma, pharyngitis, malaria, abdominal pain, insomnia, infections, and many other diseases (Evans, 1989; Blumenthal *et al.*, 2000; Wang & Nixon, 2001; Chan *et al.*, 2003). Licorice was also valued in ancient Egypt that King Tutankhamen was buried with a supply (Fleming, 2000; Hart, 2001).

Modern researches on licorice also reported its efficacy in treatment of various

diseases. Human studies proved its curative effect in infectious hepatitis (Chang, 1986; Tare *et al.*, 2005), chronic hepatitis C, and subacute hepatic failure (Acharya & Dasarathy, 1993; Fujioka *et al.*, 2003), haemophilia and HIV-1 infection (Mori, 1990; Sasaki *et al.*, 2003), gastric and duodenal ulcers (Baker, 1994), and in herpes, eczema, and psoriasis. The antibacterial effect against *Staph. aureus*, *Strept mitans*, *Mycobacterium smegmatis* and *Candida albicans* (Langmead & Rampton, 2001) was also documented. Accordingly, licorice is now used in a great variety of pharmaceutical preparations.

However, up till now, the topical effect of licorice on improvement of wound healing has not been investigated. So, the aim of the present work was to evaluate the healing effect of topically applied licorice on skin wounds using rabbits as the experimental animals.

Materials and Methods

The dried roots of licorice (purchased from Ramzy Bayrakdars and Sons, Syria) was powdered by a grinder. 100 g of the powder were placed in a beaker and 1000ml

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of ethanol 70% was added and mixed. The mixture was left at room temperature for 3 days. The extract was filtered using Whatman (No 10) filter paper and concentrated by vacuum evaporation. Creams of 5%, 10% and 15% licorice extract in eucerin (wool alcohol) were prepared. Dexpanthenol was purchased from Nile Company for Drugs, Cairo, Egypt.

Adult Newzeland rabbits of both sexes weighing 1.5-2.5 kg were used. They were housed individually at aluminium cages in room temperature around 25 °C. They had access to standard, commercial pellet diet supplemented with fresh vegetables and water *ad libitum*. The holding room was illuminated with 12 hours light/dark cycles.

A full thickness wound was made in the skin of the test animals according to the model of Cross *et al.*, 1995) as modified by Hemmati, and Mohammadian, 2000). The hair of lower back and left flank of the animals was fully shaved and locally anaesthetized using subcutaneous injection of lidocaine (2%). The animals were held in the standard crouching position, and the mobile skin of the flank was gently stretched and held by the fingers. A metal template measuring 15 x 15 mm was placed on the stretched skin and an outline of the template was traced on the skin using a fine-tipped pen. The wound was made by excising the skin, within the border of the template to the level of loose subcutaneous tissue, using a forceps and a size 15 scalpel blade. All ethical issues were considered in surgery procedure and during the treatments.

Animals were divided into six groups, each included six rabbits. Wounds of one group were left without any treatment. Eucerin base was applied to the wounds of the second group. The third group was treated topically with dexpanthenol cream as a standard healing agent. The remaining three groups were treated topically with eucerin containing licorice extract (5 %, 10% and 15%, respectively). Wound dressings were done twice daily, and bedding in the animal cages was changed

twice daily. Cages were kept clean to avoid infection of wounds.

The rate of wound healing was quantified daily. The animal was held in the standard crouching position and outline of the wound was traced on a transparent plastic sheet using a fine-tipped pen. Measurements errors were minimized by repeating each measurement three times at the same sitting and the average of this measurement was used in all calculations. The area of the wounds on the first day was considered as 100% and the wound areas on subsequent days were compared with the wound on the first day. Healing percentage was the difference between the initial wound and the healing wound.

Statistical analysis of data was done using Student's t test. A value of $p < 0.05$ was considered significant. Data are presented as means \pm standard errors (M \pm SED) of means.

Results

The rate of wound healing was nearly the same in untreated animals and animals subjected to topical application of eucerin base. In both groups healing was completed within 21 days (Fig. 1). Dexpanthenol treatment cut short the time of wound healing to 19 days (Fig 2). All three concentrations of licorice extract showed better rate of wound healing than dexpanthenol. With licorice extract creams of 5 %, 10%, and 15 %, wound healing was completed within 16, 14, and 15 days, respectively (Fig 3). Licorice cream of 10 % concentration exhibited the best healing rate. Significant differences between 10 % licorice extract-treated group and dexpanthenol-treated groups was observed (Fig. 4). The wound healing effect of 10% licorice extract cream was more pronounced compared with dexpanthenol ointment. The healing effect of the 10% concentration of licorice compared with the eucerin-treated group was also evident ($P < 0.05$) as early as the second day of treatment and continued to the end of the treatment course (Fig. 5).

Fig. 1 Untreated animals & animals subjected to topical eucerin. Values significantly different from no treatment are indicated as*(P<0.05)

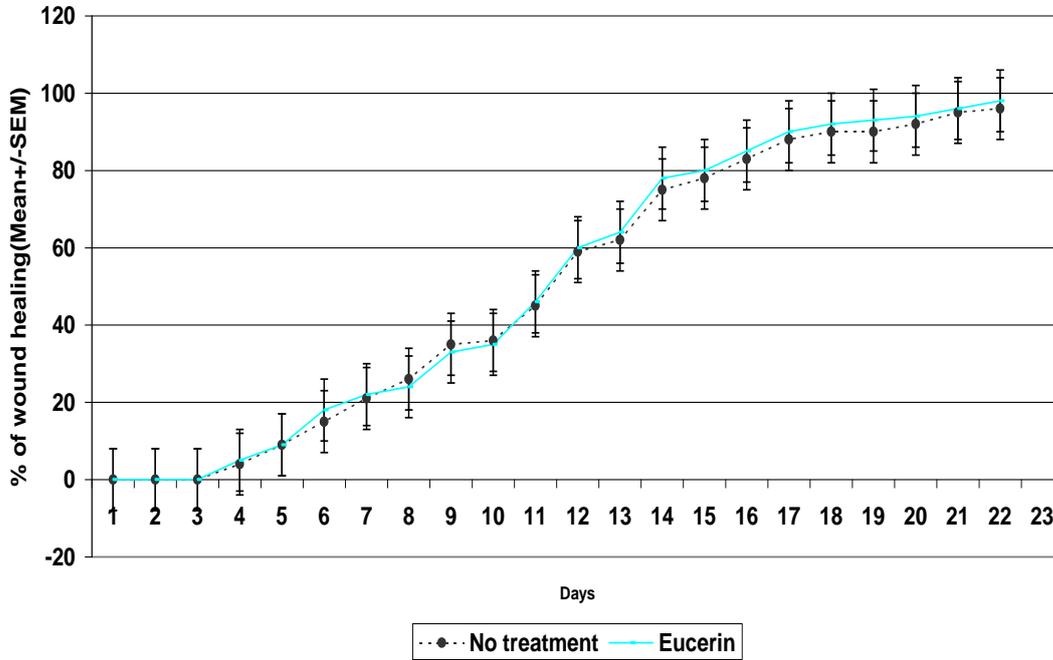
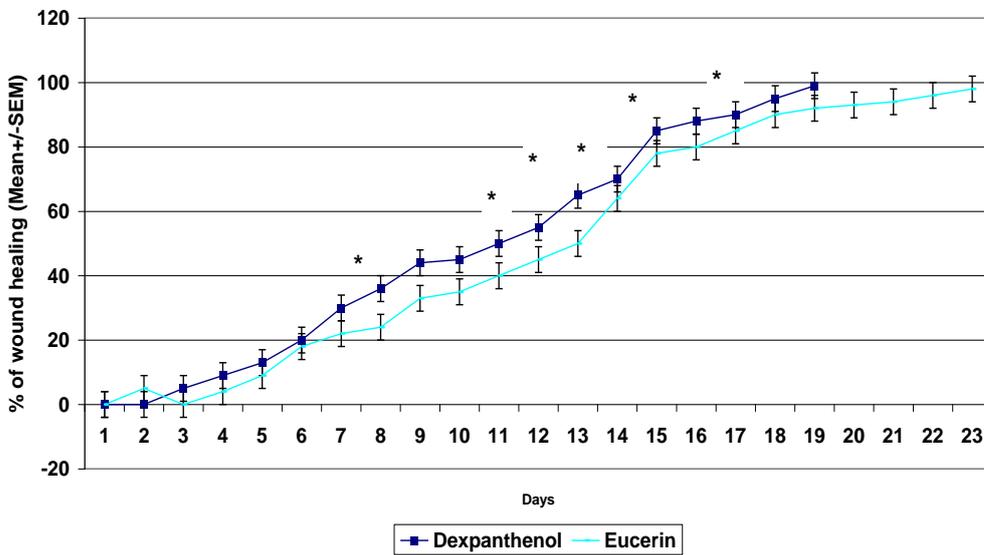


Fig. 2 Comparison of the wounds healing in eucerin & dexpanthenol treated groups. Values significantly different from eucerin are indicated as * (P<0.05) or ** (P<0.01)



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Fig. 3 Comparison of the wound healing in dexpanthenol & licorice extracts treated groups. Values significantly different indicated as * (P<0.05) or ** (P<0.01)

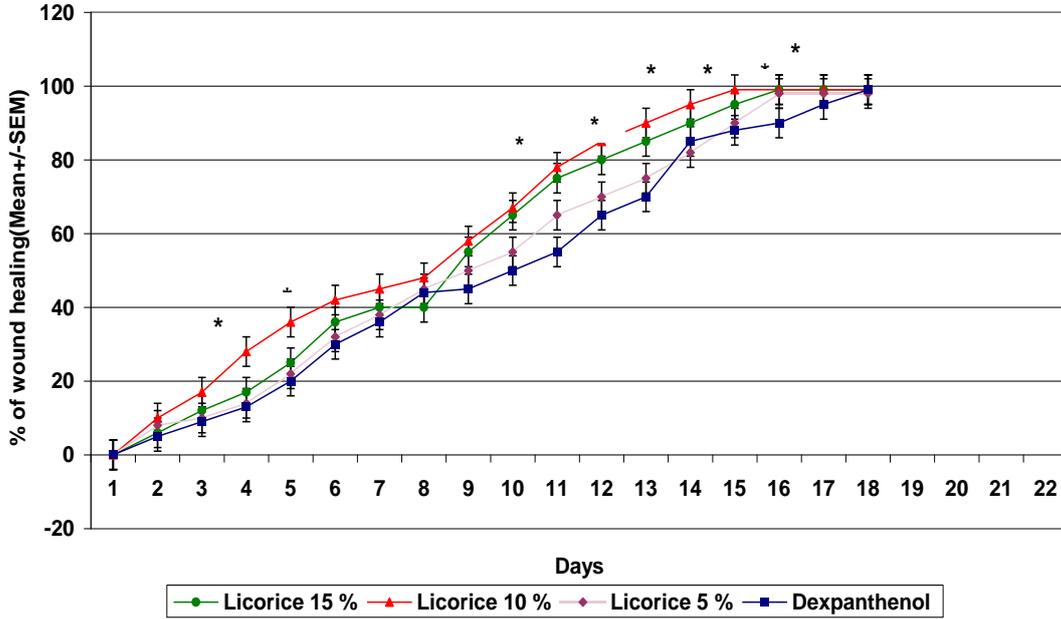


Fig. 4 Comparison of wound healing in dexpanthenol & licorice extracts cream 10 % treated groups. Values significantly different from dexpanthenol are indicated as * (P<0.05) OR ** (P<0.01)

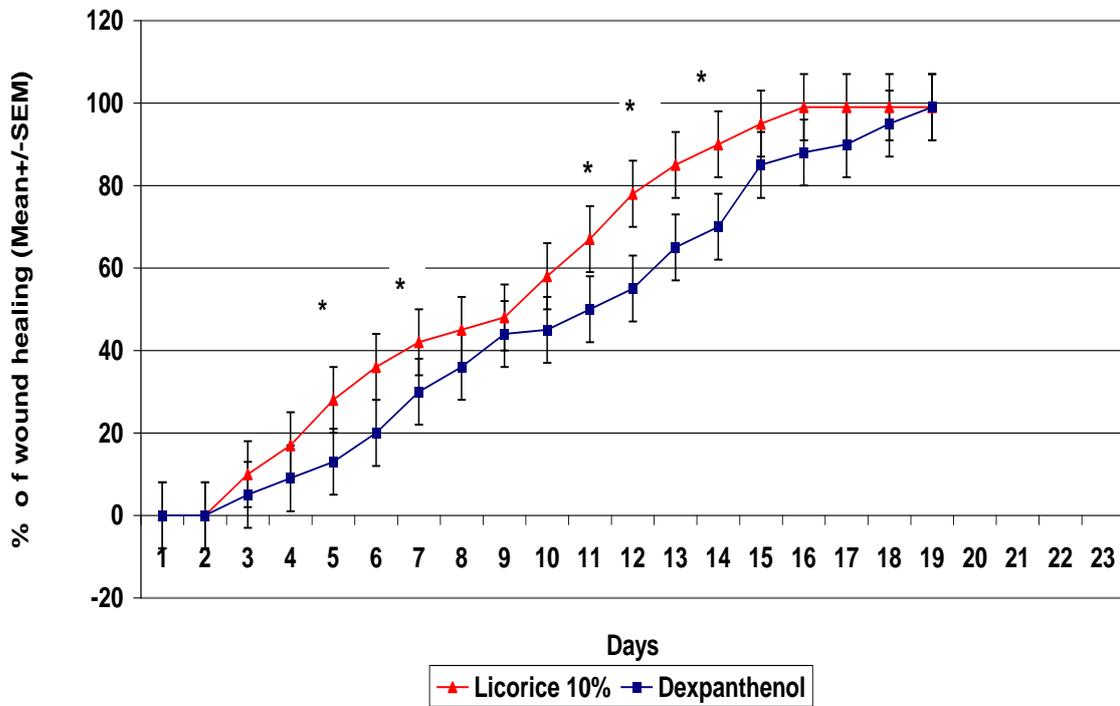
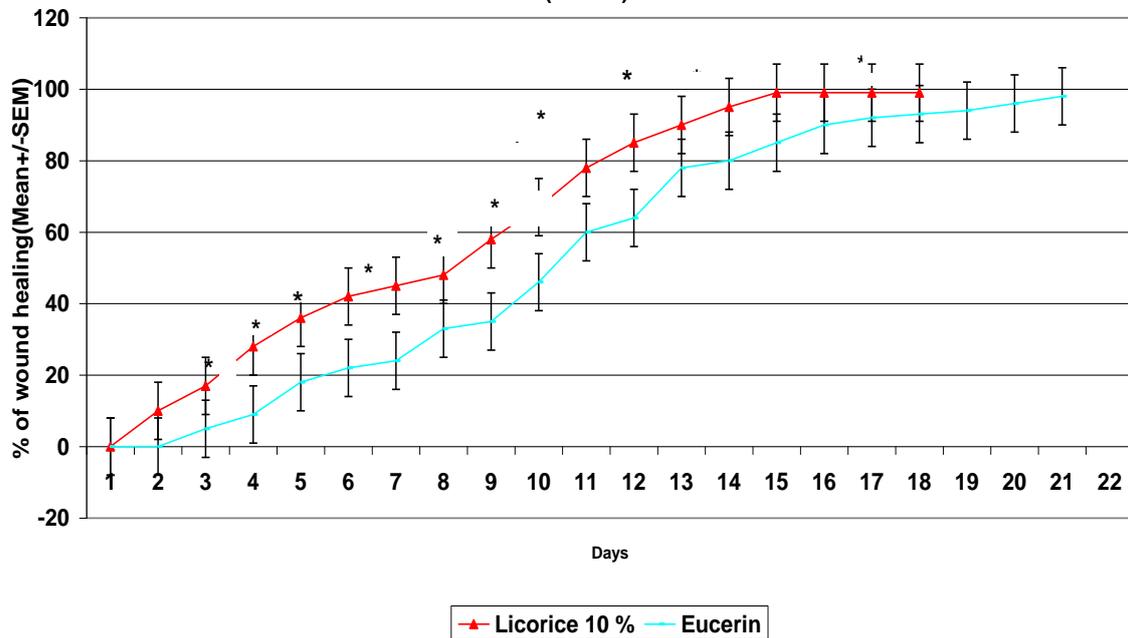


Fig. 5 : Comparison of the wound healing in eucerin & licorice extract cream 10 % treated groups. Values significantly different from eucerin are indicated as *(P<0.05) or **(P<0.01)



Discussion

Healing of wound involves various phases such as inflammation, coagulation, collagenation, wound contraction and epithelialisation. Although the coagulation and collagenation phases are interlinked, yet, wound contraction and epithelialisation are completely independent from each other but run concurrently (Bairy & Rao, 2000; Wynne *et al.*, 2004).

In the present work, an excision skin wound model for contraction and epithelialisation was employed in rabbits and the healing effect of licorice extract on the skin wound model was investigated.

The obtained results demonstrated that licorice extract reduced the time required for wound healing indicated by the faster contraction of the wound treated with licorice extract in comparison with untreated ones or those subjected to topical application of the vehicle eucerin. The percentage of healing with different concentrations of licorice extract were significantly ($p < 0.05$) higher than eucerin group from the second day of treatment until the complete closure of wound. Again licorice extract was more potent than

dexpantenol which is a standard healing agent. The 10% licorice cream was the most efficient as it cut down the duration of wound healing to 14 days only.

Although numerous studies have been done regarding the pharmacological properties of licorice (Baker, 1994; Blumenthal *et al.*, 2000; Shibata, 2000; Rotblatt & Ziment, 2004; Mike & Stew, 2005), yet, the mechanism by which this plant promotes healing has not been well documented. Few studies have been done to elucidate the mechanisms of the action of licorice on peptic and duodenal ulcers. It may increase the synthesis prostaglandin E which is protective for the gastrointestinal mucosa (Borrelli & Izzo, 2000; Langmead & Rampton, 2001; Cinati *et al.*, 2003; Lin, 2003; Fukai *et al.*, 2004). Furthermore, it has been shown that licorice may promote the production of mRNA and new proteins which are involved in the process of wound healing (Safayhi & Sailer, 1997). It is also proved that licorice has a cortisol-like effect on healing of wounds including inhibition of inflammation by inhibiting phospholipase A₂ (first step in the synthesis of

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inflammatory prostaglandins and leukotrienes) (Haraguchi *et al.*, 2000; Armanini *et al.*, 2002; Fujoka *et al.*, 2003; Hart, 2004).

Chemically, glycyrrhizin (6-10%), is the most important active ingredient in licorice besides other important constituents which include flavonoids, isoflavonoids, chalcones, coumarins, triterpenoids, sterols, starch (2-20%), sucrose, glucose (3-4%), lignins, amino acids, amines, gums, and volatile oils (Hart, 2004; Mike & Stew, 2005). Glycyrrhizin proved to increase interleukin 2 which is essential for wound healing (Acharya & Dasarathy, 1993; Yokota *et al.*, 1998; Wynne *et al.*, 2004). The isoflavonoids show in vitro activity against *Staph. aureus*, *Strept. mitans*, *Mycobacterium smegmatis* and *Candida albicans* (Shibata, 2000; Saeedi *et al.*, 2003; Mike & Stew, 2005); organisms that cause infections and delay wound healing.

However, more studies are required to elucidate the exact mechanism of licorice extract in wound healing.

Conclusion

The present work demonstrated the healing effect of licorice on wounds. Although the exact mechanism by which licorice improves wound healing is not yet clear, however, it is advisable to be used with other medications to cut short the time of wound healing and further investigations has to be done to clarify the exact mechanism by which licorice promotes wound healing.

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تأثير عرق السوس على التئام الجروح في الأرانب

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طب عين شمس***

يعتبر عرق السوس أحد النباتات الطبية الواسعة الاستخدام، ويستخدم في العديد من المستحضرات التقليدية والمستحضرات الطبية الحديثة. وقد تم في هذه الدراسة التحقق من تأثير خلاصة عرق السوس على التئام جروح الجلد المفتوحة في الأرانب، حيث استخدمت أرانب نيوزيلندا من كلا الجنسين يزن كل منهما من 1.5 – 2.5 كجم بعد حلق شعر مؤخرة الظهر والجانب الأيسر من الفخذ وإحداث جرح غائر (15 15 مم) في المنطقة المحلوقة. وتم تحضير خلاصة مائية كحولية من عرق السوس بطريقة النقع. كما تم أيضا تحضير كريمات بنسبة 5 % ، 10 %، 15% (وزن / وزن) من خلاصة العرق سوس في قاعدة الايوسرين ودهنت بها الجروح مرتين يوميا، مع استخدام كريم ديكسبانثينول كعلاج ضابط. وقورنت المجموعات المعالجة مع المجموعات غير المعالجة حيث تم تقدير الالتئام بدرجة النقص في مقاس الجرح. واكدت النتائج أن كريم عرق السوس (10 %) هو عامل التئام فعال، بل وجد أنه أفضل من كريم ديكسبانثينول.