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Wound Healing Potential of Ethanolic Extract of Roots of *Glycyrrhiza glabra* L. in Diabetes Induced Rats

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ABSTRACT

The goal of the current research was to examine the potential for *Glycyrrhiza glabra* L., (a member of the Fabaceae family) root extract to treat wounds. Using excision and burn wound models, the ethanolic root extract of *Glycyrrhiza glabra* was assessed for its wound healing efficacy in rats. When compared to the control group, which had a wound area of 28%, the extract-treated animals showed reductions in the wound area of 73% and 92%, respectively (5% and 10%w/w). In the excision model, it was discovered that extract-treated wounds epithelialized more quickly and contracted at a higher rate than control wounds. Histopathological research provided additional proof of this. Studies on wound contractions found that as the concentration of herbal extracts rises, so do the wound contractions. Both types come preinstalled with mupirocin. In diabetic rats, *Glycyrrhiza glabra* significantly speeds up wound healing; additional research into this effect in people is advised.

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INTRODUCTION

Wound healing is a natural physiological process which includes inflammation, proliferation, maturation, remodelling, and regeneration. Wounds occur when the skin is damaged due to external mechanical forces, chemical agents, radiation, thermal injury, or infections. A wound disrupts the normal continuity of the epidermis, dermis, and subcutaneous layers of the skin. In addition to these structural changes, a wound initiates a repair process involving various biochemical responses in order to restore its function [1]. The wound repair process involves three overlapping phases: hemostasis (clotting), inflammatory phase, and proliferative phase. Hemostasis occurs immediately after trauma, and it prevents blood loss, which may result in serious consequences if not treated properly. Inflammation is the primary defense mechanism of the body. During this stage, immune cells invade the area surrounding the wound, remove foreign materials, and initiate repair processes [2]. Proliferation begins approximately 10 days after wounding and continues until about 30 days. In this stage, fibroblasts and epithelial cells migrate from adjacent uninjured parts of the wounded tissue and fill the gap between injured sites. Fibroblasts produce collagen and extracellular matrix components, while

epithelial cells cover the wound surface. Finally, remodelling begins approximately 30–60 days post-wounding. During this stage, the newly formed tissues undergo modifications in shape and structure. These changes help maintain the integrity of the existing structures and facilitate tissue adaptation [3].

During wound healing, cytokines play a significant role in regulating cellular activities. Cytokines are soluble proteins produced by many types of cells and have a wide range of effects on target cells. There are two types of cytokines: proinflammatory cytokines, including interleukin (IL)-1, IL-6, tumor necrosis factor alpha, and platelet-activating factor; and anti-inflammatory cytokines, including IL-10 and transforming growth factor beta. Both types regulate and mediate the complex interactions between different cell types involved in wound healing. Cytokine production is modulated by several factors, including the type and severity of wounds, age, sex, diet, and genetic background. The cytokines that regulate wound healing are influenced by the nature of the injuries. For example, acute wounds are characterized by high levels of IL-1, IL-6 and TNF-alpha. Chronic wounds are associated with elevated levels of IL-6 and TGF-beta. In some cases, chronic wounds exhibit increased expression of both pro-inflammatory and anti-inflammatory cytokine genes. However, the balance of pro-inflammatory and anti-inflammatory cytokines appears to be critical in determining whether the wound heals normally or becomes infected [4].

Process of Wound healing

In humans, the cutaneous wound healing process consists of six distinct phases: 1) coagulation/inflammation; 2) proliferation; 3) angiogenesis; 4) remodeling; 5) maturation; and 6) regeneration.

Coagulation/Inflammation

This phase begins immediately after the injury occurs and lasts only about 30 minutes. Within this short period, blood vessels constrict and capillaries become smaller and less permeable. There is also an increase of platelets and fibrinogen at the site of injury. These substances work together to stop bleeding and prevent infection. After coagulation, inflammation begins.

Proliferation

During this phase, cells begin to multiply and migrate to repair the damaged area. Tissues at the wound site swell, which causes the skin to tighten over the wound. As the wound heals, new collagen fibers are formed around the edges.

Angiogenesis

The formation of new blood vessels at the site of the wound is called angiogenesis. Blood vessels form a network of fine tubes that supply oxygen and nutrients to cells surrounding the wound site. At first, these newly formed blood vessels are fragile and leaky. However, they eventually develop stronger walls and become fully functional. In fact, this is where the term “granulation” comes from. Granulation tissue is the mass of fibrous connective tissue and epithelial cells (skin cells) that forms over the wound [5].

Remodeling

Remodeling starts once the inflammatory stage is finished and continues until the wound is completely healed. During this time, the granulation tissue becomes thicker and tougher. Wound contraction occurs as well, which helps the wound heal faster. Remodeling involves the replacement of damaged tissue with scar tissue. Scar tissue is much denser than normal tissue and is composed mainly of collagen.

Maturation

Maturation is the final step of wound healing, and it normally takes place between two weeks and three months after the initial damage occurred. During this phase, the body prepares the wound for regeneration. Epithelial cells proliferate and start forming hair follicles and glands. The dermis thickens, and nerves sprout from the epidermis. Finally, the scar tissue matures and hardens.

Regeneration

Regeneration is the last phase of wound healing. When the wound is completely healed, the body returns to its original state. Skin grows back and becomes smooth again. New hair follicles and glands are produced, as well as sweat glands. Nerves regenerate and the dermis regains its original thickness. Finally, the scars fade away [6].

Wound Healing Potential of *Glycyrrhiza glabra* L.

Glycyrrhiza glaba L., commonly known as licorice, is a species of flowering plant in the legume family Fabaceae native to central Asia and Europe. Its root contains glycyrrhetinic acid, a substance with antifungal activity. Licorice grows best in well-drained soils rich in humus. Licorice roots are often used in herbal medicine for their sweet flavor and soothing effect. Glycyrrizin, a major compound in licorice, has several health benefits. It is a potent inhibitor of cholesterol biosynthesis (a precursor to steroid hormones) and works by inhibiting the

enzyme HMG CoA reductase. HMG CoA reduced is involved in the conversion of HMG CoA to mevalonate, which is the first step in the synthesis of cholesterol. It is also believed that licorice helps lower blood pressure and increases thyroid function. Glycyrrhizin, an active ingredient extracted from licorice root, possesses anti-inflammatory, antioxidant, and wound healing potential. Licorice root is commonly known as liquorice, sweet root, or sweet root glycyrrhiza. It has been traditionally used to treat colds, cough, asthma, bronchitis, and diabetes. It is also considered a natural remedy for skin infections and wounds due to its antibacterial activity. In addition, it is often prescribed for ulcers, mouth sores, and burns. Glycyrrhizin, glycyrrhetinic acid, and their derivatives are triterpene saponins commonly known as phytoconstituents isolated from the roots of Glycyrrhizae radix et rhizoma (licorice). Licorice root contains various active constituents including flavonoids, polysaccharides, alkaloids, saponins, sterols, tannins, and anthocyanins. These compounds have been shown to possess anti-inflammatory activity and wound healing properties in vitro and in vivo [7]. To date, no studies have been carried out to investigate the effect of ethanolic extract of glycyrrhiza on wound healing. Thus, we have chosen to study the wound healing potential of ethanolic extract from glycyrrhiza in experimental rats with excisional wounds.

Materials and Methods

Collection of Plant Material

Glycyrrhiza glabra L. (Fabaceae), was procured from the surroundings of Tirupathi and used for the present study. Roots of *Glycyrrhiza glabra* L. (Fabaceae) was collected from Tirumala hills, Tirupathi. The taxonomical identification and authentication of the plant was done by Director, National Institute of Herbal Medicine, Plant Anatomy Research Centre, Chennai.

Extraction of *Glycyrrhiza glabra* L. (Fabaceae)

Roots of *Glycyrrhiza glabra* L. were collected, washed and dried at room temperature. After complete drying, it was powdered and passed through a 60 mesh sieve and stored in air tight container. Dried powdered drug was used to prepare extract. About 50 g of air-dried powdered plant material was extracted with ethanol (60-80^{0}C), in a soxhlet apparatus [8]. Extract was filtered, the solvent was evaporated and extract was used for the study.

Animals

The study was approved by Institutional Animal Ethical Committee. Healthy Sprague Dawely rats weighing 150-180 g were maintained on the standard rodent fed and water ad libitum.

Induction of Diabetes in Rats

Rats were made diabetic by a single I.P.injection of 150mg/kg of alloxan monohydrate dissolved in saline to overnight fasted animals. It is followed by 0.5ml of 25% dextrose after 2 hours of alloxan and 5%destrose solution ad libitium for next 24 hours [9]. After 72hours of alloxan, blood samples were withdrawn from rat tail vein and blood glucose levels were estimated in all animals and animals with normal blood glucose level $\geq$200mg/dl (diabetic) were selected for study. The excision & burn wound models were used to evaluate wound healing activity of *Glycyrrhiza glabra* extract. The animals were randomly distributed into four groups of 6 each in excision and of 6 each in burn wound models.

Excision Wound Model

Animals underwent open mask diethyl ether anaesthesia before having their backs shaved using an electric clipper on both sides. Methylene blue was used to outline the intended area of the wound on the animals' backs using a stainless steel stencil. The wound was left completely open. Animals were rigorously scrutinised for any signs of infection, and those that did were removed from the trial, quarantined, and replaced [10]. Animals were divided into four groups of 6 each.

(Group 1) normal control were applied with ointment base two times a day,

(Group 2) drug treated group were applied 5 % w/w &

(Group 3) drug treated group 10%w/w ointment in soft paraffin base root extract of *Glycyrrhiza glabra* two times a day,

(Group 4) positive control group rats mupirocin ointment two times a day.

The treatment was done topically in all the cases. Wound areas were measured on days 1, 5 and 11 for all the groups using a transparency sheet and a permanent marker. Recording of the wound areas were measured on graph paper.

$$\%\ of\ wound\ closure = \frac{Wound\ area\ on\ day\ 0 - Wound\ area\ on\ day\ n \times 100}{Wound\ area\ on\ day\ 0}$$

n= numbers of days (0th, 5th, 11th).

Burn Wound Model

Animals that had been famished for the previous night were given partial thickness burn wounds by pouring hot, molten wax upon them for 10 seconds while under the anaesthetic of ketamine (100 mg/kg, i.m.) and xylazine (16 mg/kg, i.m.). The

wax was applied to the animal's shaved back using a cylinder with a 220 mm^2circular hole. It was permitted for the wax to stay on the skin until it solidified. The medications or base were topically applied both immediately following the injury and throughout the following days. Animals were divided into four groups of 6 in each group.

(Group 1) normal controls were applied with ointment base two times a day,

(Group 2) drug treated group were applied 5 % w/w &

(Group 3) drug treated group 10%w/w ointment in soft paraffin base extract of *Glycyrrhiza glabra* two times a day,

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The parameters observed in the study were as follows:

Epithelialization Period

Nothing kept track of how many days it would take for the eschar to peel away from the burn site surface without leaving a raw wound behind.

Wound Contraction

It was discovered by tracking the planimetrically progressive changes in the wound, excluding the days of wounding. The same excision wound model is used to measure wound contraction.

Histopathological Studies

On the eleventh day, the healed wounds' regenerated tissue was removed and put in 10% buffered formalin for histological examinations. Epithelization, inflammation, collagen, and fibroblasts were studied in sections from the regenerated tissues.

Statistical Analysis

Using a one-way ANOVA Post hoc test, the means of wound area measurements between groups at various time points were compared. Graph Pad software was used to compare the average differences in wound healing across the groups in excision and burn wound models [11].

RESULTS AND DISCUSSION

Glycyrrhizin (GL) is a bioactive flavonoid glucoside that is derived from licorice root extract. GL is known to have anti-inflammatory effects and is commonly used in traditional Chinese medicine for its medicinal properties. GL has been shown to reduce inflammation in skin wounds and increase collagen synthesis. In addition, GL helps regulate the immune system and decrease bacterial infection of wounds. GL is thought to act by inhibiting phosphodiesterase activity in inflammatory cells, thereby increasing cyclic AMP levels, which in turn stimulates protein kinase C activation. GL promotes fibroblast proliferation and migration, increases capillary endothelial cell growth, and stimulates angiogenesis. GL also inhibits tumor necrosis factor-alpha production by monocytes and macrophages. GL may also inhibit neutrophil chemotaxis and degranulation. GL is believed to stimulate keratinocyte differentiation, resulting in increased epidermal thickness and reepithelization. GL also enhances the rate of wound contraction and epithelialization. GL is not absorbed orally and is poorly metabolized in the body; however, it does cross the blood brain barrier and enters the central nervous system [12]. GL is generally considered safe at therapeutic doses. Licorice root extract contains high concentrations of GL and may enhance the healing effects of GL. These results suggest that licorice root extract enhanced the healing of excision wounds in rats.

Effect on Excision Wound Model

Based on these animal experiments, it can be stated that rats given root extract showed significantly increased wound healing activity.

Comparing animals from groups 1, 2, 3, and 4 to animals from groups 2, 3, and 4, they demonstrated a reduction in the epithelialization period and an increase in the proportion of wound contraction (Table 1).

In comparison to drug extract (5%), treatment with drug extract (10%) showed shorter epithelialization periods and higher wound contraction rates.

On day 11, the animals in groups 2 and 3 that had received extract showed wound contraction by 73% and 92%, respectively, compared to 28% in the control group's wounds (groups 1).

Animals treated with extract showed wound contraction outcomes that were similar to those of positive controls (95%). Comparative investigation showed that the high dose of root extract (at 10%) was much more effective than the low dose (at 5%) at shortening the epithelialization period.

Effect on Burn Wound Model

Similar to the excision wound model, topical treatment of 5% and 10% formulations of *Glycyrrhiza glabra* root extract and mupirocin standard medica-

Table 1: Effect of *Glycyrrhiza glabra* Root Extracton %Wound Contraction and Epithelization Period in Excision Wound

Group	Day 1	Day 5	Day 11	Period of Epithelization in (days)
Group-1	216 ± 1.905	12.6 ± 3.5	29.3 ± 4.6	22.12 ± 0.3
Group-2	215.3 ± 1.635	43.3 ± 1.5	74.9 ± 2.9	18.4 ± 4.8
Group-3	211 ± 1.850	53.9 ± 3.7	91.8 ± 2.6	15.3 ± 3.1
Group-4	217.6 ± 1.878	63.8 ± 1.4	97.6 ± 1.9	13.6 ± 2.8

Shows Significant as Compared to Normal Control ($p<0.001$) [Values are mean ± SE from 6 rats in each group]

Table 2: Effect of *Glycyrrhiza glabra* Root Extract on %Wound Contraction, Days of 50% Wound Contraction and Epithelization Period in Burn Wound

Group	Day 1	Day 5	Day 11	Wound Contraction 50% (days)	Period of Epithelization in (days)
Group-1	218 ± 1.980	13.6 ± 3.5	18.9 ± 3.6	8.84 ± 0.421	41.12 ± 0.3
Group-2	217.4 ± 1.645	9.3 ± 1.6	28.9 ± 2.9	6.94 ± 0.368	40.4 ± 4.8
Group-3	216 ± 1.860	9.9 ± 3.5	33.8 ± 2..6	6.13 ± 0.362	30.2 ± 2.1
Group-4	218.6 ± 1.978	13.8 ± 1.6	42.9 ± 11.9	5.12 ± 0.135	26.2 ± 1.8

Shows Significant as Compared to Normal Control ($p<0.001$) [Values are mean ± SE from 6 Rats in Each Group]

tion resulted in considerable wound contraction of 50% (days) as compared to control.

Comparative study of the various groups reveals that the high dose of leaf extract was more successful than the low dose in shortening the epithelialization period (Table 2).

Histopathological Studies

The following characteristics were observed during microscopic analysis of the histopathological examinations performed on the sections taken from the wounds of the normal, control, and treatment groups.

Normal

Blood arteries, fibroblasts with round to oval nuclei, and thick collagen fibres make up the tissue.

Control

Incomplete wound healing was seen in the tissue's heavily inflamed connective tissue and the presence of chronic inflammatory cells between the collagen fibres. There are a lot of blood vessels with thin walls.

Groups Treated

Tissues had fibrous connective tissue and fibroblasts were dispersed throughout the tissues. Progressive collagenation was present, and there were few blood vessels with thin walls and little lumina. Tissues undergoing epithelialization were seen. The treated group had larger levels of fibroblasts, collagen, and neovascularization than the control group did. These findings demonstrate that the treated group's wound healing was quicker than that of the control group. Higher extract concentration formulations (5% & 10%) revealed dense fibrous tissue with thick collagen bundles, fibroblasts, and sporadic inflammatory cells. Its look was remarkably similar to that of healthy tissues. In the image below, normal, control, and wounds treated with the ointment-based formulation's regenerated tissue sections are all compared using histological investigations (Figure 1).

To investigate the effects of topically applying *Glycyrrhiza glabra* root extract on wound healing and contraction, an excision wound model was used. Rats treated with root extract showed increased wound healing activity. Animals from group 4 displayed a higher proportion of wound contraction on the fifth day when compared to animals from all other groups. On the eleventh day, the same pattern was seen as well. Animals treated with extract had wound contraction results that were comparable to those of successful controls. Studies on wound contraction showed that when the concentration of the herbal extract increases, the wound contraction increases. The rats treated with the root extract ointment preparation in the current study showed a significantly better burn wound contraction. It can be argued that *Glycyrrhiza glabra* extract could be an inexpensive and efficient adjuvant to other topical treatments for achieving faster, less complication-filled wound healing. Numerous research studies

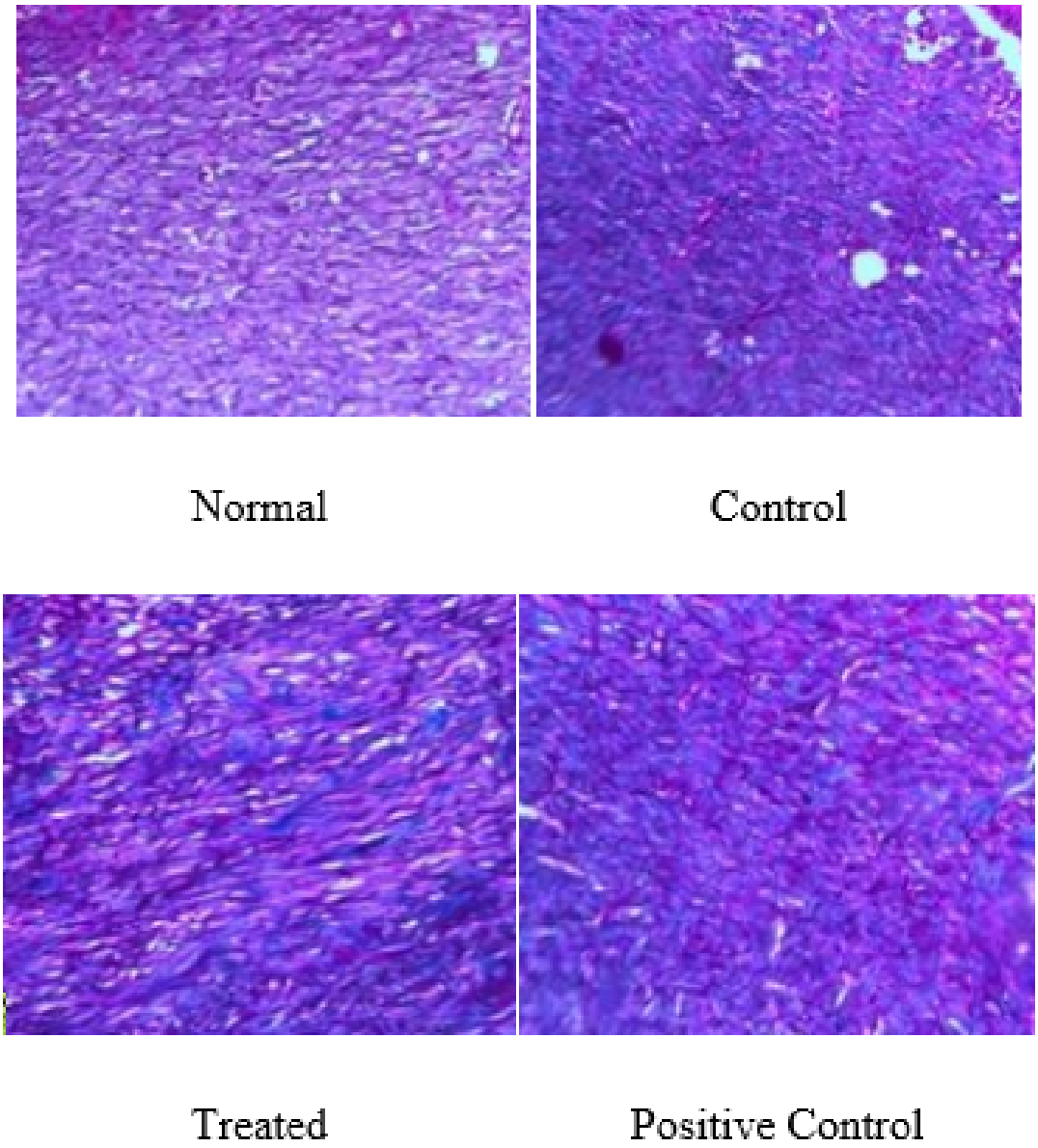

Figure 1: Histology of Regenerated Tissue of Open Wounds in Normal, & Treated Group as on the 11^{th} Day

have been conducted in the past on the use of natural remedies for the treatment of burn wounds, but these were primarily focused on infection management. The anti-inflammatory properties of several natural products may also aid in the recovery of burn wounds. Although the precise method of healing of *Glycyrrhiza glabra* was not investigated in the current study, it could be ascribed to both anti-inflammatory and antibacterial qualities. Based on these, we suggest that *Glycyrrhiza glabra* extract could greatly expand the range of topical burn treatments that are now offered. The results of the histological studies indicated that the treated groups of animals had somewhat higher levels of collagen, fibroblasts, and neovascularization than the control groups, indicating that the wounds had healed more quickly.

The aforementioned observations lead us to the conclusion that these topical formulations with herbal extract have potent wound-healing properties.

CONCLUSION

The current work shows that topically applied *Glycyrrhiza glabra* root extract enhances wound contraction during healing in excision and burn wound models. These preliminary findings also imply that *Glycyrrhiza glabra* promotes healing by speeding up and extending wound closure.

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Conflict of Interest

The authors declare that there is no conflict of interest for this study.

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