Research Paper

Effect of Glycyrrhizic Acid and Curcumin on Wound Healing in Streptozotocin-induced Diabetic Mice

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ABSTRACT

Introduction: Diabetes mellitus is a prevalent global metabolic disorder characterized by insulin resistance and pancreatic beta-cell dysfunction. Among its most severe complications are vascular disorders and the development of diabetic ulcers, particularly in the lower extremities. This study aimed to examine the effects of a combination therapy of curcumin and glycyrrhizic acid on diabetic wound healing in a murine model.

Methods: Type 2 diabetes was induced in animals by administering a high-fat diet (4900 kcal/kg) for 21 days, followed by a single intraperitoneal injection of streptozotocin (35 mg/kg). Serum glucose and insulin levels were measured using a glucometer and ELISA kit, respectively. Histopathological analysis was conducted using hematoxylin and eosin and Masson's trichrome staining.

Results: Macroscopic evaluation showed that the average wound closure rate was significantly lower in the control group compared to all treatment groups. A reduction in the wound area was observed in the groups treated with silver sulfadiazine, glycyrrhizic acid, and curcumin. Histopathological assessment indicated enhanced re-epithelialization and granulation tissue formation at the wound site in the groups treated with glycyrrhizic acid, curcumin, or silver sulfadiazine. Contrary to expectations, however, the combination of glycyrrhizic acid and curcumin did not produce synergistic effects on the regeneration rate or collagen deposition.

Conclusion: Glycyrrhizic acid and curcumin accelerated diabetic wound closure in mice. This effect is mediated through modulation of the inflammatory phase and promotion of epidermal regeneration. However, the combination of the two compounds did not yield synergistic improvement in wound healing outcomes.

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Introduction

Diabetes mellitus is characterized by insulin resistance and pancreatic beta-cell dysfunction. In the disease's early stages, beta cells compensate for insulin resistance by increasing insulin secretion to maintain glucose homeostasis. However, prolonged insulin resistance leads to a progressive decline in beta-cell function. This dysfunction ultimately results in chronic hyperglycemia and disrupts lipid metabolism [1]. Infection is a primary complication in wounds and is responsible for approximately 51% of patient mortality in this context [2]. Diabetic patients are at a heightened risk of microvascular disease, which can impair localized blood flow at the wound site. Hyperglycemia exacerbates this by increasing basement membrane permeability and further disrupting circulation, leading to significant delays in wound healing [3]. Therefore, controlling inflammation and accelerating wound closure are critical therapeutic strategies to reduce infection risk in this patient population.

Curcumin is a lipophilic polyphenol with antioxidant and anti-inflammatory properties. Studies have shown that curcumin exerts anti-inflammatory effects by inhibiting the synthesis of inflammatory prostaglandins. Curcumin has the potential to eliminate free radicals and inhibit oxidative stress [4]. Glycyrrhizic acid is one of the main saponins of licorice root. Its structure and anti-inflammatory action are similar to steroids such as cortisol [5]. Glycyrrhizic acid accelerates wound healing by promoting angiogenesis via multiple molecular pathways. Studies have shown that glycyrrhizic acid increases the expression of the anti-inflammatory factor transforming growth factor beta (TGF-β1) and decreases the expression of matrix metalloproteinase-9 (MMP9) in skin keratinocytes through the Nrf2 pathway, thereby stimulating angiogenesis in wounds [6].

This study aimed to investigate the effects of curcumin and glycyrrhizic acid on wound healing in diabetic mice.

Materials and Methods

Animals

This study utilized eight-week-old male mice weighing 22–25 g each. Animals were housed under controlled environmental conditions, with temperature maintained at 22±2 ˚C and a 12-hour light/dark cycle. All experimental protocols were conducted in accordance with standard guidelines for the care and use of laboratory animals.

Diabetic model protocol

In the present study, a high-fat diet and streptozotocin (STZ) were used. First, the mice were fed a high-fat diet (60% of calories from fat) for 3 weeks. Then, they were administered a single dose of STZ (35 mg/kg) intraperitoneally [7]. After 72 h, blood glucose levels were measured using a glucometer. Animals with blood glucose greater than 250 mg/dL were considered diabetic models. Blood insulin was also measured using an ELISA kit to confirm the induction of type-2 diabetes.

Incisional wound model

Wound-healing procedures using biopsy punches were performed as described previously. In this study, diabetic wounds were induced by shaving the dorsal fur of the anesthetized animals and creating a full-thickness wound using a sterile 5 mm biopsy punch. A silicone splint was then placed around the wound and secured to the underlying tissue with 5-0 surgical sutures to prevent wound contraction [8]. The mice were randomly allocated to five experimental groups. The first group served as a wound control, consisting of diabetic mice with induced wounds that received no further treatment. In the second and third groups, diabetic wounds were treated with curcumin (Nano Exir Co, Iran) and glycyrrhizic acid (Shirin Darou Co, Iran), respectively. In the fourth group, silver sulfadiazine ointment was used as a routine wound treatment. In the fifth group, diabetic wounds were treated with curcumin and glycyrrhizic acid.

Macroscopic wound analysis

To evaluate wound size changes, the wound was captured from day 0 to the end of the study under the same conditions for all groups. To accurately measure the wound size, a calibrated ruler was placed next to the wound, and ImageJ software, version 1.54i was used to quantify the wound size.

Histopathological analysis

Full-thickness skin samples were gathered from all groups. The samples were then washed with physiological serum and immediately placed in 10% formalin for fixation. Then, tissue processing was performed, and 5-µm serial sections were prepared. Histopathological changes in tissue were assessed by hematoxylin and eosin staining. To evaluate the maturity of skin connective tissue, Masson's trichrome staining was performed. With Masson's trichrome stains, the collagen, nuclei, and epithelium are stained in blue, black, and pale blue, respectively.

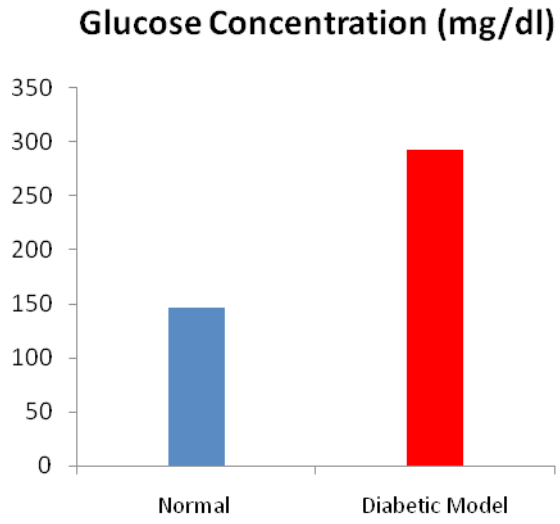

Figure 1. Serum glucose levels in normal and diabetic mice

Statistical analysis

Data are expressed as the Mean±SD. Statistical analyses were performed using one-way analysis of variance followed by a post hoc t-test. A $P<0.05$ was considered statistically significant.

Results

The results indicated that the serum glucose levels in diabetic mice (293±21 mg/dL) were higher than those in the normal group (147±12 mg/dl) (Figure 1). Insulin concentrations in diabetic and normal mice were 198±26 and 255±9 pmol/L, respectively. The results showed a decrease in blood insulin levels in diabetic rats compared to the normal group (Figure 2).

The macroscopic results showed that the average contracting speed of the control group was much slower than that of the treatment groups. The rate of wound closure was significantly higher with glycyrrhizic acid and silver sulfadiazine than with curcumin or the combination treatment.

Figure 3 shows the results of hematoxylin and eosin staining. A pronounced accumulation of inflammatory cells was observed in the wound control group. In contrast, both the glycyrrhizic acid- and silver sulfadiazine-treated groups exhibited reduced inflammatory infiltration at the wound site. Re-epithelialization progressed effectively in these treatment groups. Furthermore, granulation tissue formation was increased across all treatment groups, with these increases being significant relative to the control. Notably, the organization of the newly formed epidermal layer was more orderly in wounds treated with silver sulfadiazine and glycyrrhizic acid than in those treated with other groups.

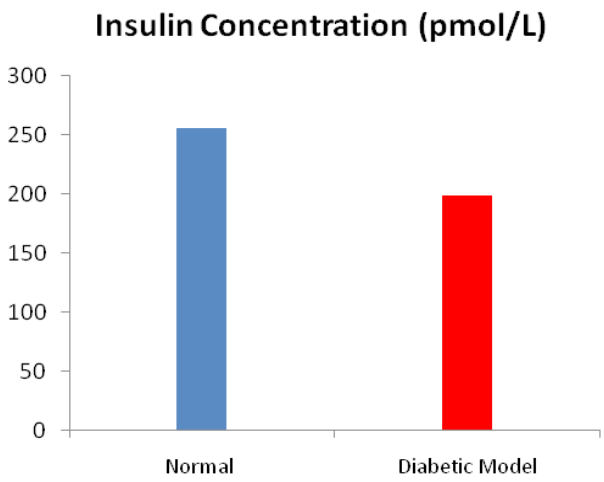

Figure 2. Insulin concentrations in normal and diabetic mice

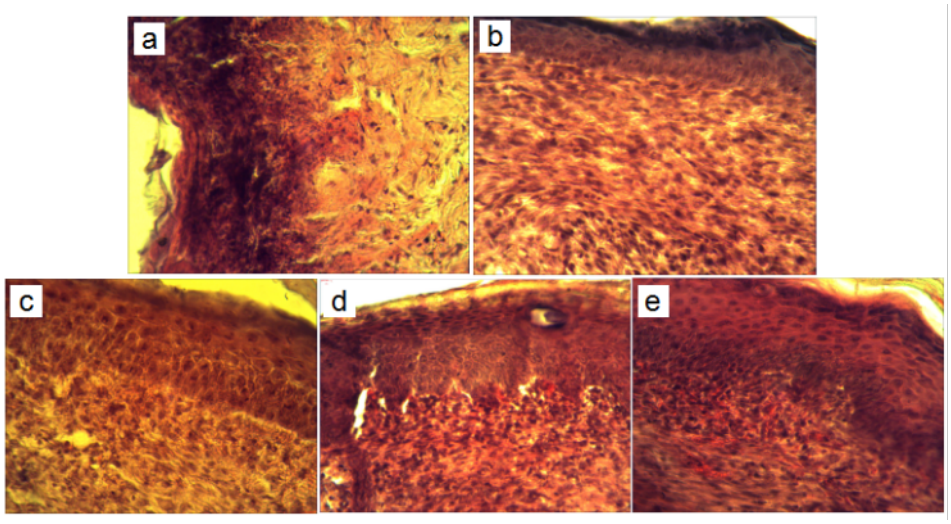

Figure 3. Hematoxylin-eosin staining of the wound area in the studied groups
a) Control group, b) silversulfadiazine treatment, c) treatment with glycyrrhizic acid, d) curcumin treatment, e) combined treatment with curcumin and glycyrrhizic acid.

Results from Masson's trichrome staining indicated that collagen deposition was more pronounced in wounds treated with glycyrrhizic acid or silver sulfadiazine compared to other treatment groups. Furthermore, the architecture and alignment of collagen fibers appeared more organized and regular in the glycyrrhizic acid-treated group (Figure 4). The green color represents collagen fibers.

Discussion

The results of this study demonstrated that both glycyrrhizic acid and curcumin accelerated diabetic wound closure in mice. Histopathological analysis further revealed that these compounds promoted epidermal regeneration and enhanced collagen deposition at the wound site. Notably, glycyrrhizic acid induced more pronounced improvements in overall healing progression and collagen organization compared to curcumin. By facilitating rapid re-epithelialization and wound contraction, these treatments may help mitigate severe complications of diabetic wounds, such as infection.

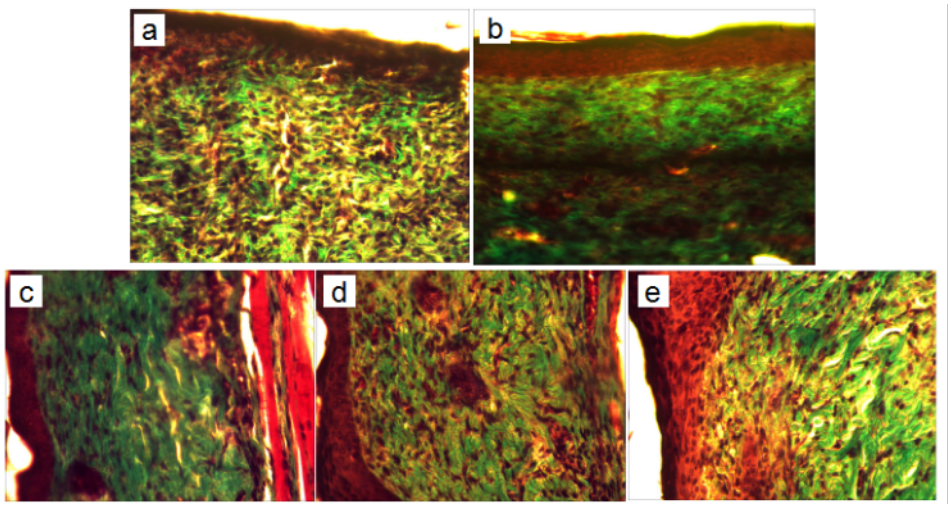

Figure 4. Masson's trichrome staining of the wound area in the studied groups
a) Control Group, b) silversulfadiazine treatment, c) treatment with glycyrrhizic acid, d) curcumin treatment, e) combined treatment with curcumin and glycyrrhizic acid.

Our findings align with those of Oloumi et al. [9], who examined the wound-healing properties of licorice root extract in rats. Their study reported that aqueous licorice extract significantly increased fibroblast and capillary bud counts, hydroxyproline content, and wound tensile strength, while reducing wound area compared to the control group. Similarly, Salehi et al. [10] demonstrated that an alcoholic extract of curcumin accelerated diabetic wound healing in male Wistar rats compared to untreated controls. These results are consistent with the improved wound closure observed in the present study.

We hypothesized that the combination of curcumin and glycyrrhizic acid would exert a synergistic effect, increasing the rate of diabetic wound healing. However, our study found that their combined use does not increase the rate of wound closure, epidermal regeneration, or collagen production in the diabetic wound bed. Based on our study, glycyrrhizic acid and curcumin significantly increased wound-healing speed, though glycyrrhizic acid has greater effects due to its stronger anti-inflammatory properties. Because curcumin is poorly soluble, rapidly metabolized, and toxic at high doses, it is highly dose-dependent and has short-term effects.

Conclusion

Both glycyrrhizic acid and curcumin independently accelerate diabetic wound closure in mice. This effect is likely mediated by their ability to modulate inflammation and promote epidermal regeneration.

Ethical Considerations

Compliance with ethical guidelines

The study was approved by the Ethics Committee of Baqiyatallah University of Medical Sciences, Tehran, Iran (Code:IR.BMSU.REC.1400.040).

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Authors' contributions

Study design and Original draft preparation: Bahman Jalali Kondori; Data collection: Fateme azemati and Asgar Moghani; Software, validation and formal analysis: Seyed Morteza Hosseini; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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