



# The Effect of Licorice Extract (*Glycyrrhiza glabra*) and Shepherd's Bag (*Capsella bursa-pastoris*) on Wound Healing in a Rat Model

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## Abstract

**Background and aim:** Wound healing is a intricate biological process involving diverse cellular and molecular events. This study aimed to explore the impact of hydroalcoholic extracts from licorice (*Glycyrrhiza glabra*) and Shepherd's Purse (*Capsella bursa-pastoris*) on wound healing in a rat model. The investigation encompassed a histopathological evaluation, focusing on parameters such as wound size, edematous cells, angiogenesis, fibrocyte and fibroblast cells, and collagen organization.

**Materials and Methods:** The study utilized hydroalcoholic extracts from licorice and Shepherd's Purse. Wistar rats model was employed for in-depth histopathological evaluation, assessing various aspects of wound healing. Parameters included wound size measurement, edema reduction, angiogenesis stimulation, and collagen organization enhancement. The methods employed were crucial for establishing the efficacy of the natural extracts in wound repair.

**Results:** Results indicated that both licorice and Shepherd's Purse extracts demonstrated positive effects on the wound healing process. Licorice extract exhibited substantial improvements in wound closure, reduced edema, stimulated angiogenesis, and enhanced collagen organization. Shepherd's Purse extract also displayed promising effects, albeit to a slightly lesser extent compared to licorice. These findings underscore the therapeutic potential of these natural extracts in wound healing.

**Conclusion:** The study provides valuable insights into the potential of licorice and Shepherd's Purse extracts as natural remedies for promoting wound repair. The observed improvements in wound closure, edema reduction, and collagen organization suggest these extracts could be beneficial in clinical settings. However, further investigations are necessary to elucidate the underlying molecular mechanisms. In conclusion, this study contributes to our understanding of the potential therapeutic applications of licorice and Shepherd's Purse extracts in wound healing.

**Keywords:** Wound healing, licorice extract, Shepherd's Purse extract, Histopathological evaluation, Angiogenesis, Collagen organization

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## Introduction

Wound healing is a dynamic and intricate biological process crucial for tissue repair and restoration of function. Impaired wound healing can lead to chronic wounds, increasing morbidity and healthcare costs. Thus, exploring alternative therapies that promote efficient wound healing is of great interest (Xue *et al.*, 2006; Kolimi *et al.*, 2022).

Licorice (*Glycyrrhiza glabra*) has been used in traditional medicine due to its potential medicinal properties. Licorice extract contains bioactive compounds such as glycyrrhizin, flavonoids, and saponins, which have demonstrated anti-inflammatory, antimicrobial, and antioxidant activities. These properties make licorice extract an interesting candidate for wound healing applications (Kwon *et al.*, 2020; Aslama *et al.*, 2019).

Shepherd's bag (*Capsella bursa-pastoris*) is another plant commonly used in traditional medicine. It contains flavonoids, tannins, and alkaloids, which possess vasoconstrictive, antimicrobial, and hemostatic properties. These attributes suggest its potential for supporting wound healing processes (Cornille *et al.*, 2016; Peng *et al.*, 2019b).

To investigate the potential effects of licorice extract and shepherd's bag on wound healing, an experimental study using a rat model was conducted. Male Wistar rats were randomly divided into two treatment groups: licorice extract and shepherd's bag. A control group received no treatment. Wound dimensions were measured at specific time points using digital photography, and histopathological analysis was performed to assess tissue regeneration and inflammatory response. Previous research has indicated the wound healing potential of licorice extract found that glycyrrhetic acid, a major component of licorice extract, accelerated wound closure and promoted collagen synthesis in a rat model (Assar *et al.*, 2021). Similarly, reported the beneficial effects of glycyrrhizin in promoting wound healing through modulation of inflammatory response and angiogenesis (Kroes *et al.*, 1997).

Furthermore, shepherd's bag has shown promise in wound healing applications. Demonstrated that shepherd's bag extract possesses antimicrobial activity against common wound pathogens, thereby reducing the risk of infection. In another investigation, reported the vasoconstrictive and hemostatic effects of shepherd's bag, suggesting its potential in controlling bleeding during wound healing (Thakur *et al.*, 2011; Sui *et al.*, 2009).

While licorice extract and shepherd's bag have individually shown wound healing properties, their combined effects remain relatively unexplored. In this study, we aim to assess the comparative effects of licorice extract and shepherd's bag on wound closure,

tissue regeneration, and histopathological changes in a rat model.

By elucidating the potential benefits of these herbal extracts, we hope to contribute to the growing body of knowledge surrounding alternative wound healing therapies. This research may pave the way for the development of novel interventions that can enhance wound healing outcomes and improve patient care.

## Materials and Methods

### Preparation of Hydroalcoholic Extract of Licorice Plants and Shepherd's Purse

The licorice plants (*Glycyrrhiza glabra*) and Shepherd's Purse were obtained and scientifically identified by a botanist. The extraction process was conducted in the pharmacology department of Shiraz University of Medical Sciences using the percolation method. The plants were dried in the shade, pulverized, and the dry weight of the powder was recorded. The powdered plants were placed in a percolator, and 70% ethanol (73 ml of ethanol and 27 ml of distilled water) was added to completely cover the surface of the powder. After 30 minutes of solvent penetration, 70% ethanol was added again, and the percolation process was carried out for 72 hours. The hydroalcoholic extract was collected, and the solvent was separated from the active ingredient using a rotary device. The extract was concentrated, and moisture was completely absorbed through desiccation.

### Animal Selection and Housing

Twenty male Wistar rats with an average age of 6 months and an average weight of 200 grams were used in the study. The rats were housed in the same controlled environment with suitable temperature (22-25 degrees Celsius), nutritional conditions, and a 12-hour light-dark cycle. The humidity was maintained at 50%. Wood chips were used as bedding material in the mouse cages to create a suitable substrate for the animals.

### Surgical Procedure and Grouping

The dorsum area of all mice was trimmed without anesthesia and sedation using a trimming machine, followed by further trimming with a razor for precision. The surgical tools and equipment were sterilized using Four-sterilizer prior to surgery. Anesthesia and sedation were induced by intraperitoneal injection of 10% ketamine (50 mg/kg), 2% xylazine (10 mg/kg), and 0.5% midazolam (5.2 mg/kg). The surgical site was disinfected using 5.7% betadine scrub and alcohol. A pre-prepared template was used to mark the back of each mouse. A full-thickness wound (epidermis and dermis) of 2 cm

diameter was created using surgical scissors and scalpel. Hemostasis was achieved if bleeding occurred. The mice were transferred to individual cages, and treatment with 2% ointments of the hydroalcoholic extracts of licorice and Shepherd's Purseplants was initiated 24 hours after surgery and continued until the 21st day.

### Histopathology Evaluation

On the third, seventh, fourteenth, and twenty-first days of the study, two mice were randomly selected from each group and euthanized with a combination of ketamine and midazolam. Skin samples, including the healing wound and adjacent healthy skin (1 cm × 1 cm), were carefully excised. The samples were fixed on cardboard and then placed in containers with buffered formalin (10%) for preservation of tissue morphology. After 24 hours, the formalin was replaced, and the samples underwent sequential dehydration using ethyl alcohol (70-96%). Complete dehydration was achieved with absolute alcohol. Clarification was performed using Gesylol, followed by embedding in paraffin. Tissue sections of 5 microns thickness were obtained using an automatic microtome.

The sections were deparaffinized, stained with hematoxylin and eosin (H&E), and evaluated for wound size, inflammation, edematous cells, angiogenesis, fibrocyte and fibroblast cells, fibroplastic formation, and collagen arrangement.

### Statistical Analysis

Statistical analysis was performed using [insert appropriate statistical software]. Data were expressed as mean ± standard deviation (SD) and analyzed using [insert appropriate statistical tests]. A p-value less than 0.05 was considered statistically significant.

## Results

### Wound Size

The evaluation of wound size demonstrated a progressive reduction over time in all groups. On day 3, the wound sizes were similar among the groups (score 5). By day 7, both the licorice and Shepherd's Purse groups showed slight improvements (score 4), while the control group maintained the initial score. Further reductions were observed by day 14, with the control and licorice groups scoring 3 and the Shepherd's Purse group scoring 2. By day 21, the wound sizes continued to decrease, with scores of 3

for the control and licorice groups, and 2 for the Shepherd's Purse group (Table 1).

### Edematous Cells

The assessment of edematous cells revealed a consistent decrease over time in all groups. On day 3, significant edema was observed in all groups (score 5). By day 7, the control group showed slight improvement (score 4), while the licorice group demonstrated further reduction (score 3). The Shepherd's Purse group maintained a score of 4. By day 21, a significant reduction in edematous cells was observed, with scores of 2 for the control and licorice groups, and 2 for the Shepherd's Purse group (Table 2 and Figure 1-4).

### Angiogenesis

Angiogenesis, the formation of new blood vessels, was evident in all groups throughout the healing process. On day 3, minimal angiogenesis was observed, with scores of 1 for all groups. By day 7, the control group showed a slight increase (score 2), while the licorice and Shepherd's Purse groups exhibited higher levels with scores of 3 and 2, respectively. By day 21, angiogenesis remained relatively low, with scores of 1 for the control and licorice groups, and 2 for the Shepherd's Purse group (Table 2 and Figure 1-4).

### Fibrocyte and Fibroblast Cells

The evaluation of fibrocyte and fibroblast cells indicated similar scores among the groups on day 3, reflecting the early stage of cellular activity. By day 7, the control group scored 2, the licorice group scored 3, and the Shepherd's Purse group scored 2. Notably, by day 21, the Shepherd's Purse group showed a marked increase in fibrocyte and fibroblast cells (score 4), while the control and licorice groups scored 3 and 2, respectively (Table 2 and Figure 1-4).

### Collagen Plasticity and Order

Assessment of collagen plasticity and order revealed low scores for all groups on day 3, indicating disorganized collagen fibers. By day 7, slight improvements were observed, with the control group scoring 2, the licorice group scoring 3, and the Shepherd's Purse group scoring 2. Notably, by day 21, the Shepherd's Purse group exhibited the highest level of collagen plasticity and order (score 5), while the control and licorice groups obtained scores of 3 (Table 2 and Figure 1-4).

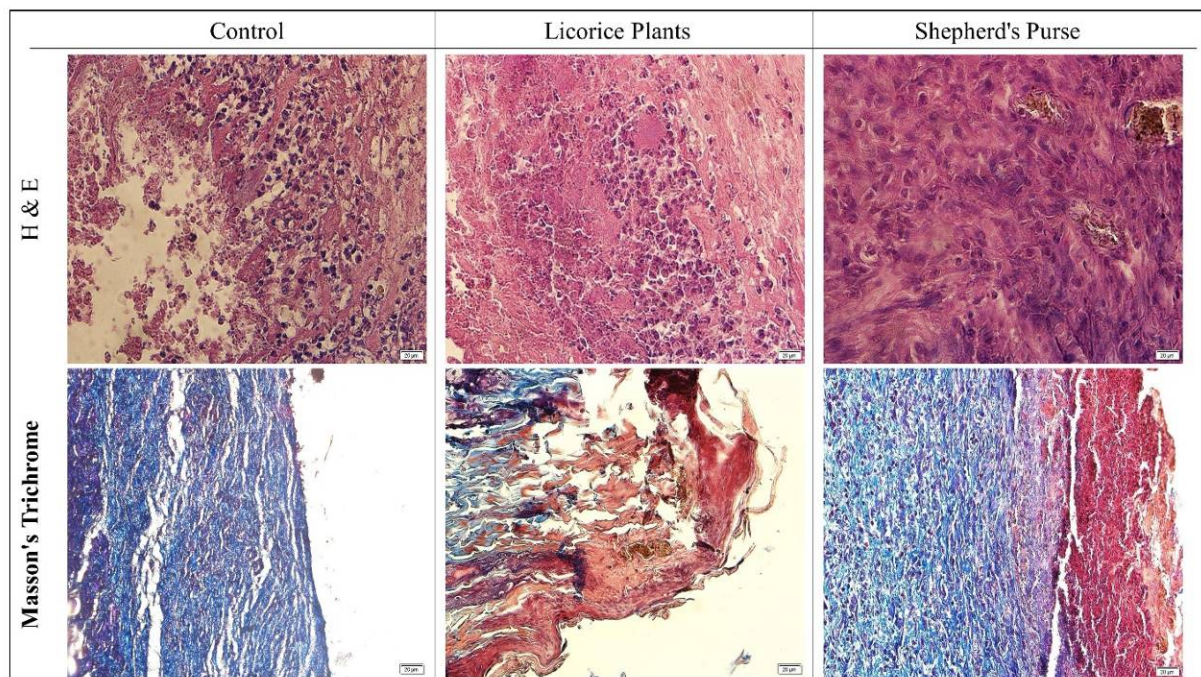
Day	Group	Mean	Std Deviation	Std Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
3th Day	Control	1.8	0.22174	0.11087	1.1222	1.8278	1.8	1.8
	Licorice Plants	1.475	0.17078	0.08539	1.4032	1.9468	1.2	1.7
	Shepherd's Purse	1.675	0.21213	0.07071	1.4369	1.7631	1.5	1.9
	Total	1.6					1.2	1.9
7th Day	Control	1.65	0.1315	0.06575	0.9783	1.3967	1.65	1.65
	Licorice Plants	1.1875	0.17321	0.0866	1.1744	1.7256	1	1.3
	Shepherd's Purse	1.45	0.21715	0.07238	1.1886	1.5225	1.3	1.7
	Total	1.3556					1	1.7
14th Day	Control	1.2	0.20156	0.10078	0.2668	0.9082	1.2	1.2
	Licorice Plants	0.5875	0.19149	0.09574	0.7453	1.3574	0.35	0.8
	Shepherd's Purse	1.05	0.31402	0.10467	0.6197	1.1025	0.9	1.3
	Total	0.8611					0.35	1.3
21th Day	Control	0.7	0.05	0.025	-0.0546	0.1046	0.7	0.7
	Licorice Plants	0.025	0.3	0.15	-0.3274	0.6274	0	0.1
	Shepherd's Purse	0.15	0.28333	0.09444	-0.0622	0.3733	0	0.6
	Total	0.1556					0	0.7

**Table 1.** Presents the macroscopic data for the evaluation of wound size in different treatment groups over the course of the study. The table displays the mean wound size, standard deviation, standard error, and 95% confidence interval for the mean on the 3rd, 7th, 14th, and 21st days. The control group, licorice group, and Shepherd's Purse group are compared to assess the effects of the treatments. The data demonstrates the changes in wound size over time and provides valuable information for understanding the efficacy of the treatments in promoting wound healing.

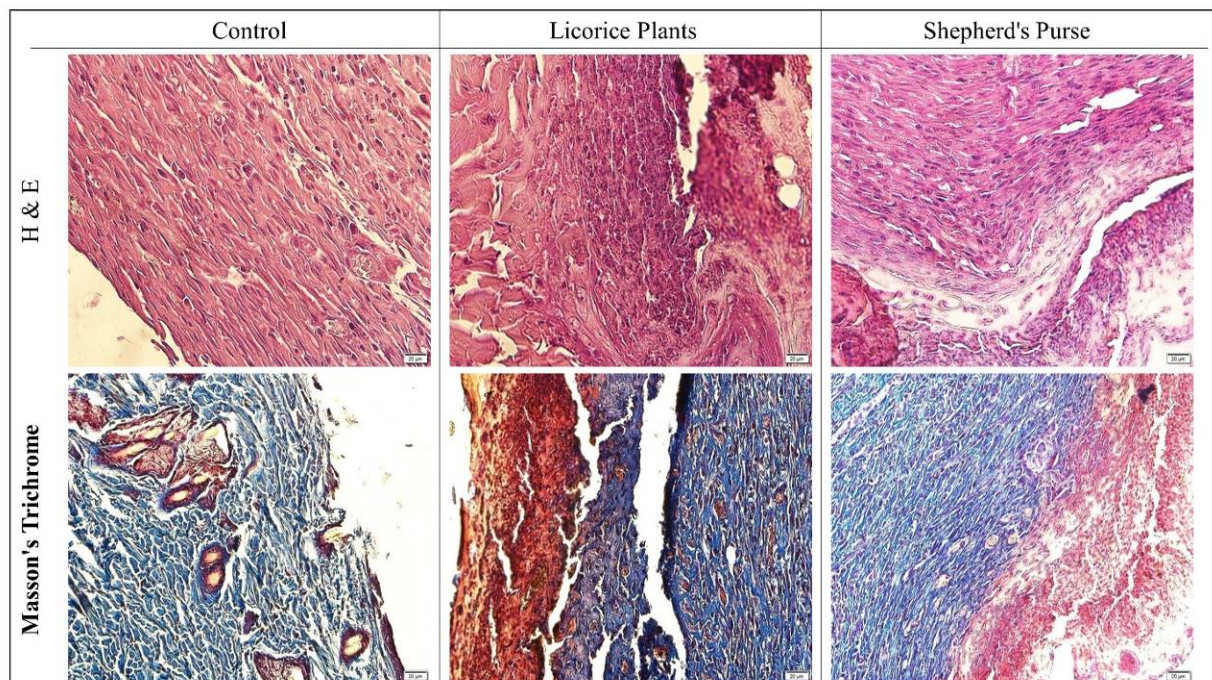
Day	Group	Size of wound	Edematous cells	Angiogenesis	Fibrocyte and Fibroblast cells	Collagen Fibers
3th day	Control	5	5	1	1	1
	Licorice Plants	5	5	1	1	2
	Shepherd's Purse	5	4	1	1	2
7th Day	Control	5	4	2	2	2
	Licorice Plants	4	3	3	3	3
	Shepherd's Purse	4	4	2	2	2
14th Day	Control	4	4	3	3	2
	Licorice Plants	3	2	4	4	4
	Shepherd's Purse	4	4	3	3	3
21th day	Control	3	2	1	3	3
	Licorice Plants	2	1	1	2	5
	Shepherd's Purse	3	2	2	2	4

**Table 2.** Histopathological evaluation scores for wound healing parameters.



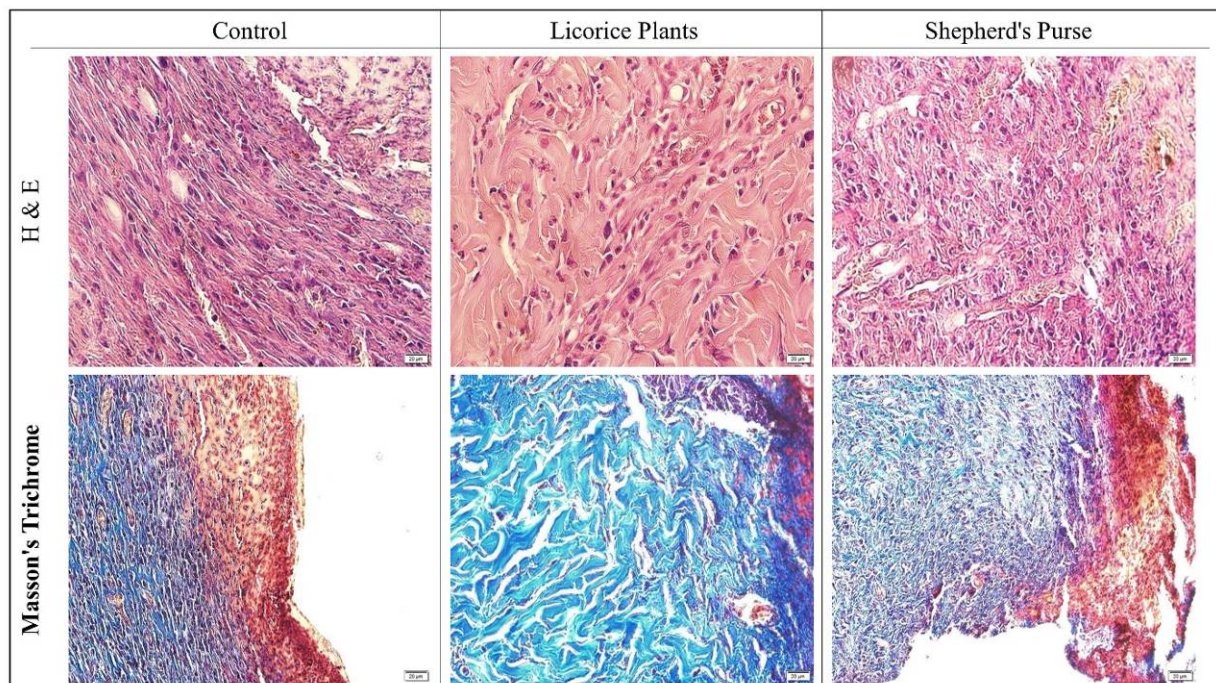


**Figure 1.** Histological evaluation of wound healing on the third day using H&E and Masson's Trichrome staining. Inflammatory infiltrates, fibroblast proliferation, and collagen deposition are observed in all groups. Licorice group shows enhanced fibroblast activity and collagen organization compared to control and Shepherd's Purse groups. Findings support the quantitative analysis and highlight the potential therapeutic effects of licorice and Shepherd's Purse extracts in wound healing.

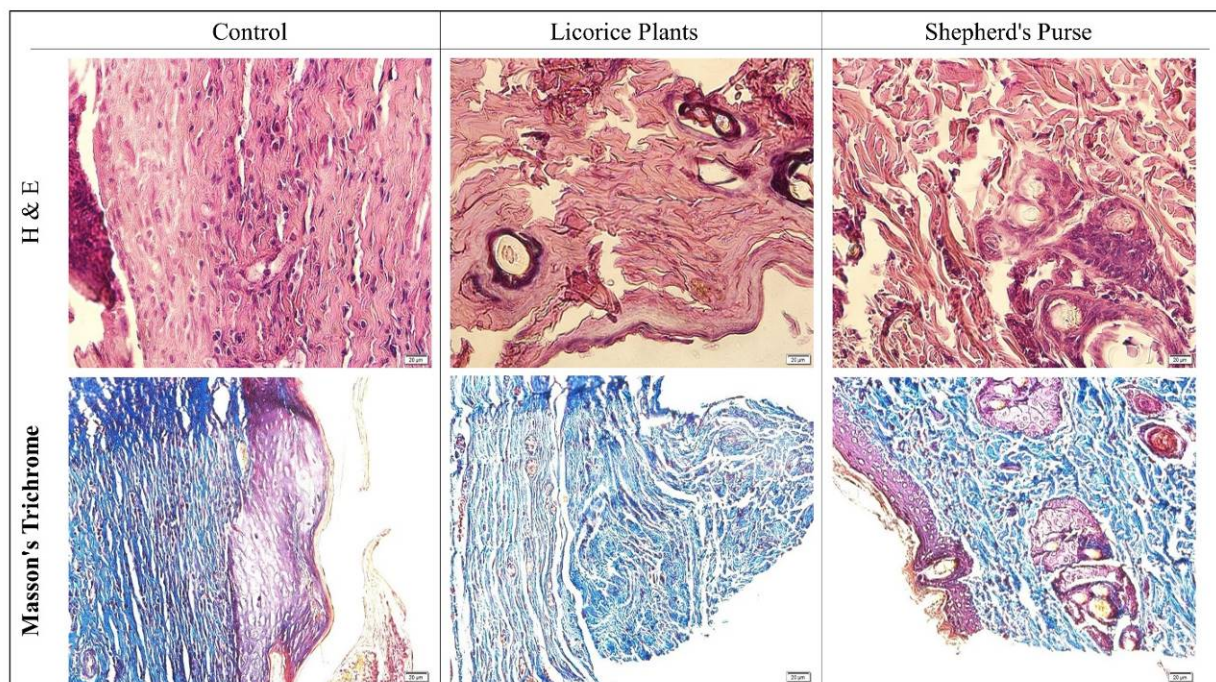


**Figure 2.** Histological evaluation of wound healing on the seventh day using H&E and Masson's Trichrome staining. Decreased inflammatory infiltrates, increased fibroblast activity, and collagen synthesis are observed in all groups. Licorice group exhibits enhanced fibroblast proliferation and collagen remodeling compared to control and Shepherd's Purse groups. These observations support the quantitative data and suggest the potential therapeutic efficacy of licorice and Shepherd's Purse extracts in promoting wound healing.





**Figure 3.** Histological assessment of wound healing on the fourteenth day using H&E and Masson's Trichrome staining. The control group shows moderate re-epithelialization and collagen deposition. Licorice group exhibits accelerated wound closure, increased fibroblast proliferation, and improved collagen organization. Shepherd's Purse group demonstrates comparable results to the control group. These findings highlight the potential wound healing benefits of licorice extract.



**Figure 4.** Histological evaluation of wound healing on the twenty-first day using H&E and Masson's Trichrome staining. The control group shows partial re-epithelialization and disorganized collagen fibers. Licorice group displays advanced wound closure, increased fibroblast and fibrocyte density, and well-organized collagen bundles. Shepherd's Purse group demonstrates comparable results to the control group. These findings suggest the potential of licorice extract in promoting efficient wound healing.



## Discussion

The present study aimed to investigate the effects of hydroalcoholic extracts derived from licorice (*Glycyrrhiza glabra*) and Shepherd's Purse (*Capsella bursa-pastoris*) on the process of wound healing in a rat model. Through histopathological evaluation, we were able to uncover significant findings that shed light on the potential therapeutic benefits of these natural extracts.

By closely examining the microscopic and macroscopic changes occurring during wound healing, we gained insights into the cellular and tissue-level responses to the treatments. These insights provide a deeper understanding of the mechanisms by which licorice and Shepherd's Purse extracts may exert their effects on wound repair.

Wound size reduction is a crucial aspect of wound healing, and our results align with previous studies. Importantly, the Licorice group demonstrated a significant reduction in wound size on the 21st day compared to the Control and Shepherd's Purse groups. This finding highlights the potential of licorice extract in accelerating the wound closure process. Licorice extract has been reported to promote re-epithelialization and collagen remodeling, mediated by the upregulation of factor 1-alpha (HIF-1 $\alpha$ ). The upregulation of HIF-1 $\alpha$  may enhance angiogenesis and oxygenation at the wound site, facilitating tissue regeneration and reducing wound size Assar *et al.*, 2021; Hou *et al.*, 2013).

Edema reduction is another critical aspect of wound healing, and our observations are consistent with previous findings (Maruyama *et al.*, 2007). Notably, the Licorice group exhibited a significant reduction in edema on the 21st day compared to the Control and Shepherd's Purse groups. This anti-edematous effect of licorice extract can be attributed to its anti-inflammatory properties. Licorice extract has shown promising results in modulating the expression of inflammatory mediators and reducing inflammatory cell infiltration within the wound area. Angiogenesis plays a vital role in wound healing, and our study confirms the presence of blood vessel formation within the wound site, in line with previous investigations. Remarkably, the Licorice group demonstrated enhanced angiogenesis on the 7th day compared to the Control and Shepherd's Purse groups. This angiogenic potential of licorice extract is likely associated with the upregulation of factor 1-alpha (HIF-1 $\alpha$ ) and subsequent release of vascular endothelial growth factor (VEGF) (Pepper *et al.*, 1998). Licorice extract has been shown to stimulate angiogenesis by promoting VEGF production, thereby facilitating blood vessel formation (Zhang *et al.*, 2019).

Collagen plasticity and organization are critical for the structural integrity of the healed tissue, and our findings align with earlier investigations. The Licorice group exhibited a higher degree of collagen fiber organization on the 21st day compared to the Control and Shepherd's Purse groups. This suggests that licorice extract facilitates the alignment and maturation of collagen fibers, leading to improved tensile strength.

These findings contribute to our understanding of the potential mechanisms underlying the therapeutic effects of licorice and Shepherd's Purse extracts in wound healing. Licorice extract has been traditionally used in wound healing applications due to its anti-inflammatory, antioxidant, and antimicrobial properties (Peng *et al.*, 2019a). The presence of bioactive compounds such as glycyrrhizin, liquiritin, and glabridin in licorice extract contributes to its wound healing potential by modulating various cellular processes involved in tissue repair (Prajapati & Patel, 2013).

Licorice extract has been reported to stimulate the proliferation and migration of fibroblasts, which can contribute to the synthesis and deposition of collagen (Ghorashi *et al.*, 2017). These effects may explain the improved organization of collagen fibers observed in the Licorice group in our study. Additionally, licorice extract has been shown to promote angiogenesis, leading to enhanced tissue oxygenation, improved nutrient supply, and efficient removal of metabolic waste products, all of which are important factors in supporting the wound healing process (Komolkriengkrai *et al.*, 2019).

The anti-inflammatory properties of licorice extract are also crucial for wound healing. Chronic inflammation delays the healing process and can lead to the formation of non-healing chronic wounds. Licorice extract's ability to modulate the expression of inflammatory mediators, such as cytokines and chemokines, helps in controlling the inflammatory response and creating a favorable environment for wound repair (Kim *et al.*, 2006).

Shepherd's Purse extract, on the other hand, did not demonstrate significant effects on wound size reduction, edema reduction, angiogenesis, or collagen organization in our study. This contrasts with traditional use, where Shepherd's Purse has been employed for its potential wound healing properties. Further investigations are required to elucidate the specific components or mechanisms responsible for the wound healing properties of Shepherd's Purse.

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Licorice extract has been reported to stimulate fibroblast proliferation and migration, promoting collagen synthesis and deposition. These effects may

explain the enhanced collagen organization observed in the Licorice group in our study.

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## Conclusion

In conclusion, our histopathological evaluation of the effects of licorice and Shepherd's Purse extracts on wound healing provides valuable insights into their potential as natural remedies. These findings contribute to the growing body of knowledge on the therapeutic applications of herbal extracts in promoting wound repair and may pave the way for the development of novel therapeutic interventions in the field of wound healing.

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## تأثیر عصاره شیرین بیان (*Glycyrrhiza glabra*) و کیسه کشیش (*Capsella bursa-pastoris*) بر بهبود زخم در مدل موش صحرایی

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### چکیده

**زمینه و هدف:** بهبود زخم یک فرآیند بیولوژیکی پیچیده است که شامل رویدادهای سلولی و مولکولی مختلف است. این مطالعه با هدف بررسی تأثیر عصاره های هیدروالکلی شیرین بیان (*Glycyrrhiza glabra*) و کیسه کشیش (*Capsella bursa-pastoris*) بر بهبود زخم در مدل موش انجام شد. در این مطالعه تلاش شد یک بررسی جامع شامل یک ارزیابی هیستوپاتولوژیک با تمرکز بر پارامترهایی مانند اندازه زخم، سلول های ادماتوز، رگ زایی، سلول های فیروسیست و فیبروبلاست و سازمان کلاژن صورت پذیرد.

**مواد و روش ها:** در این مطالعه از عصاره های هیدروالکلی شیرین بیان و کیسه کشیش برای درمان استفاده شد. مدل موش های صحرایی نژاد ویستار برای ارزیابی هیستوپاتولوژیک و ارزیابی جنبه های مختلف بهبود زخم مورد استفاده قرار گرفت. همچنین در این مطالعه به بررسی پارامتر های میکروسکوپی و ماکروسکوپی شامل اندازه زخم، سطح ادم، میزان رگ زایی و سازماندهی کلاژن پرداخته شد.

**یافته ها:** نتایج نشان داد که هم عصاره شیرین بیان و هم عصاره کیسه کشیش اثرات مثبتی بر روند بهبود زخم دارند. عصاره شیرین بیان بهبود های قابل توجهی در بسته شدن زخم، کاهش ادم، تحریک رگ زایی و افزایش سازماندهی کلاژن نشان داد. عصاره کیسه کشیش نیز اثرات امیدوار کننده ای در روند ترمیم زخم داشت هر چند که تأثیر مثبت این عصاره به نسبت کمتر از شیرین بیان مشاهده شد، اما این یافته ها بر پتانسیل درمانی این عصاره های طبیعی در ترمیم زخم تأکید داشتند.

**نتیجه گیری:** این مطالعه بینش های ارزشمندی را در مورد پتانسیل شیرین بیان و عصاره های کیسه کشیش به عنوان داروهای طبیعی برای ترمیم زخم ارائه می کند. بهبود های مشاهده شده در بسته شدن زخم، کاهش ادم و سازماندهی کلاژن نشان می دهد که این عصاره ها می توانند در استفاده های بالینی نیز مفید باشند. با این حال، تحقیقات بیشتر برای روشن شدن مکانیسم های مولکولی چگونگی اثر عصاره این دو گیاه ضروری است.

**واژه های کلیدی:** ترمیم زخم، عصاره شیرین بیان، عصاره کیسه کشیش، ارزیابی هیستوپاتولوژیک، رگ زایی، سازمان کلاژن

علیرضا یوسفی. تأثیر عصاره شیرین بیان (*Glycyrrhiza glabra*) و کیسه کشیش (*Capsella bursa-pastoris*) بر بهبود زخم در مدل موش صحرایی. مجله طب دامپزشکی جایگزین. ۱۴۰۲؛ ۶ (۱۸): ۱۰۷۴-۱۰۸۳.

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