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Evaluation of the Effect of Locally Delivered *Portulaca Oleracea* (Purslane) and *Foeniculum Vulgare* (Fennel) Extract for Deep Second-Degree Burns in A Rat Model

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Abstract

BURN wounds represent a significant challenge in clinical care, and the search for effective treatments continues to be a priority. This study investigates the wound healing potential of Foeniculum vulgare (fennel) and Portulaca oleracea (purslane) for deep second-degree burns in a rat model. Hydrogel formulations containing fennel or purslane extracts were applied to burn wounds, and their effects on wound closure were evaluated. The results showed that both fennel and purslane treatments significantly accelerated wound healing compared to the silver sulfadiazine and control groups. with fennel demonstrating the most pronounced effects. Fennel-treated wounds exhibited complete re-epithelialization, organized collagen deposition, and minimal inflammation, while purslane-treated wounds showed moderate improvement with partial epithelial regeneration. Meanwhile, silver sulfadiazine treatment resulted in re-epithelialization but did not promote the same level of tissue maturation. These findings highlight fennel and purslane as promising natural alternatives for burn wound management, with fennel showing superior therapeutic effects in promoting wound healing and tissue regeneration.

Keywords: Burn, Portulaca oleracea, Foeniculum vulgare, hydrogel, inflammation.

Introduction

One type of tissue damage brought on by exposure to thermal, electrical, chemical, or radiation energy is called a burn. Based on the anatomical structures implicated and the extent of tissue destruction, burn severity is categorized. Burns are classified clinically into first-, second-, third-, and fourth-degree categories [1, 2].

One of the first physiological reactions to a burn injury is acute inflammation, which manifests as localized erythema and edema [3]. In severe cases, the inflammatory cascade may become prolonged, potentially leading to systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS). While inflammation is essential for wound healing—mediated by cytokines and growth factors—dysregulated or prolonged inflammation can contribute to fibrosis [4].

Cutaneous burn wound healing is a complex process involving the coordinated interaction of cellular populations, biochemical mediators, and the extracellular matrix (ECM). The extent of the burn directly impacts the level of inflammation, skin regrowth, and scarring. Superficial first-degree burns usually involve minimal inflammation and insignificant scarring, while deep second- and third-degree burns are linked to substantial inflammation, pronounced scarring, and potential functional complications [5]. The healing process is regulated by mitogenic and chemotactic factors that mediate interactions between inflammatory cells, biochemical mediators, and ECM components [6, 7].

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Burn wound healing occurs via primary or secondary intention, depending on the depth and severity of the injury. This process consists of several temporally and spatially regulated stages, including inflammation, angiogenesis, fibrogenesis, and regeneration.

Despite advances in burn care, optimizing wound healing remains a significant clinical challenge. Since the 1970s, topical antimicrobial agents have been a cornerstone of burn management. Research on cutaneous wound healing continues to be a major focus due to its substantial clinical, scientific, and economic implications [8].

Portulaca Oleracea., commonly known as purslane, is a widely distributed medicinal plant that has a long history of use as both a food source and a traditional remedy for various ailments. This plant thrives in tropical and subtropical regions, including the United States. Culinary applications of purslane include using it as a potherb, in soups and salads, and as a pickle. The succulent stems and leaves of the plant can be consumed raw or cooked, and they provide a rich source of omega-3 fatty acids and antioxidants. Traditionally, purslane has been used for its fever-reducing, antiseptic, and deworming properties, as well as for treating gastrointestinal, liver, and stomach disorders. Pharmacological studies have demonstrated that purslane has antibacterial, anti-ulcer, anti-inflammatory, antioxidant, and wound-healing effects, making it a promising candidate for the management of burn wounds [9].

Foeniculum vulgare., commonly known as fennel, is a flowering medicinal plant from the Apiaceae family, originating in the Mediterranean region. It has since naturalized in various parts of the world, particularly in arid coastal and riparian environments. Fennel has been used in Mediterranean folk medicine for centuries, primarily for its essential oil's pharmacological properties. It has been traditionally employed as a carminative, digestive aid, and diuretic. Additionally, fennel is known to enhance connective tissue elasticity and has anti-aging properties. Commercial formulations incorporating fennel essential oil are available. Its documented anti-inflammatory, antimicrobial, and antioxidant properties suggest potential applications in burn wound healing [10].

This study aimed to evaluate the efficacy of *Foeniculum vulgare* and *Portulaca oleracea* in promoting cutaneous wound healing of deep second-degree burns in a rat model. Specifically, it assessed the impact of these botanical agent extracts on wound closure kinetics in healthy rats.

Material and Methods

Plant materials and extract preparation

Fennel Extraction

The net-dried fennel seeds were quickly washed twice with double distilled water and then soaked in 75% ethanol for 48 hours at 4°C. The mixture was filtered twice using filter paper to obtain the leachate. Subsequently, the leaching solution was transferred to the oven. The ethanol was evaporated through a 40°C temperature for 12 hrs to obtain the dark yellow extract. After this post-processing step, the bioactive materials were transferred to the lyophilizer for the freeze-drying process [11].

Purslane Extraction

Purslane was thoroughly washed twice using double distilled water, then underwent solvent extraction using a 3:1 mixture of ethanol and acetone at a temperature of 70°C for a duration of 5 hours. This process was aimed at isolating the bioactive compounds present in the plant. Subsequently, the leaching solution was transferred to an oven set at 40°C for 12 hrs to obtain the dark green extract. Finally, the bioactive materials were subjected to a freeze-drying process in a lyophilizer for post-processing [12].

Hydrogel Preparation

Weigh 3 gm of carboxymethyle cellulose powder was dissolved in deionized water heated 40°C under constant stirring until a homogeneous solution was obtained then slowly 1gm of crosslinking citric acid agent was added while stirring temperature was adjusted at 90°c. Additionally, 0.5 gm Mannitol was added as preservative. After that 1 gm of dried material purslane or fennel was added each one separately [13].

Animals and experimental design

Twenty-eight female Wistar rats, aged 10 weeks and weighing 150 ± 20 gm, were obtained from the Animal House Colony of the Tanta Center. The animals were provided a standard diet (El-Anani Co., Dakahlia, Egypt) and had free access to water. To prevent biting and potential wound scratching among the rats, each one was housed individually in a separate plastic cage. The rats were maintained under standard controlled environmental and nutritional conditions, and were allowed one week to acclimate before the start of the experiment. The environmental parameters were as follows: temperature 22–25 °C, relative humidity 50–60%, and a 12-hour photoperiod (lights on from 07:00 to 19:00 hr). The study protocol was approved by the research ethics committee at the Faculty of Veterinary Medicine, Kafr Elesheikh University, Egypt.

The burn model was conducted in a manner consistent with previous studies, which involved the creation of deep second-degree burns using metal bars heated to 90–100 °C for 10–15 seconds [14, 15]. Specifically, a metal bar measuring 2x2 cm was heated in boiling water until it reached a temperature of 90 °C. The rats were then anesthetized with a combination of Ketamine (70 mg/kg) and Xylazine (7 mg/kg) [16] the hair on their backs was shaved then contacted with the heated metal bar for 12 seconds. The burn area was subsequently covered with a wound dressing (Band-Aid Advanced Healing, Tri M strip), which was changed every three days (on days 3, 6, 9, 12, 15, 18, and 21). The dressing was fixed in place using medical tape and was applied while the animals were under anesthesia.

All 28 rats were randomly divided into 4 groups, with 7 rats in each group. The first group was the control group that received no treatment. The second group was treated with a 1% Silver Sulphadiazin ointment (Silvirburn, Medical Union Pharmaceuticals, Egypt). The third group was treated with a hydrogel containing Purslane. The fourth group was treated with a hydrogel containing Fennel.

Assessment of the burn area

The burn area size was photographed and measured using specialized size analysis software, NIH Image J, which was downloaded from (http://www.rsb.info.nih.gov/ij). This was done on days 0, 3, 6, 9, 12, 15, 18, and 21 after the burn incident. The change in wound size was then expressed as a percentage relative to the original wound size (day 0).

Tissue collection

At the end of the study animals were euthanized by an overdose of pentobarbital anesthesia (500 mg/kg) administered via intraperitoneal injection. Samples were fixed in 10% neutral buffer formaldehyde for histological examination.

Histopathological examination

Cutaneous tissue samples were collected from different experimental groups and fixed in 10% neutral buffered formalin for preservation. After fixation, the tissues were processed through standard dehydration and clearance protocols before being embedded in paraffin. The embedded tissues were then sectioned into 5 µm thick slices using a microtome. For histopathological analysis, the sections were stained with hematoxylin and eosin (H&E) to evaluate general tissue morphology. The stained sections were examined under a light microscope to assess histological changes and tissue structure.

Statistical analyses

Statistical analyses were carried out using one-way and repeated measures two-way ANOVA, followed by Tukey's post-hoc test in GraphPad Prism 10. A P-value of less than 0.05 was deemed statistically significant.

Results

Wound area

During the initial three-day period following burn injury, all experimental groups developed necrotic eschar, with no observable differences among them. However, by day 15, both the purslane- and fennel-treated groups exhibited significantly accelerated wound closure compared to the control and silver sulfadiazine 1% groups.

Wound areas were normalized to baseline measurements recorded on day 3 (set at 100%). Throughout the observation period (day 6 to day 21), both treatment groups demonstrated consistently enhanced wound area size relative to controls. On day 15, purslane-treated wounds showed a 42.41% ± 2.54 wound size, while fennel-treated wounds showed a 35.47% ± 2.19 wound size. In contrast, the control and silver sulfadiazine 1% groups exhibited wound size of 59.87% ± 2.27 and 62.27% ± 3.19, respectively ($p < 0.001$).

Importantly, complete wound closure was achieved earlier in all rats treated with purslane and fennel compared to both control groups (Fig. 1 and 2).

Histopathological Assessment of Burn Wound Healing

In the control non-treated group (Fig. 3 A, A 1), sections showed a thick necrotic scab (SC) covering the wound with complete loss of the epithelial layer. The underlying granulation tissue (GT) was immature and characterized by edema with clefts between loosely arranged collagen fibers and extensive inflammatory cell infiltration (black arrowheads). In some cases, fibrin deposition and deep necrosis were evident. While, the silver sulfadiazine 1% treated group (Fig. 3 B, B 1) demonstrated marked re-epithelialization (EP) and a significant reduction in necrotic scab formation. The underlying granulation tissue was more organized, showing dense cellularity, perpendicular blood capillaries, and moderate inflammatory infiltration (white arrowhead), indicating ongoing healing. In the purslane-treated group (Fig. 3 C, C 1), a partially continuous epithelial tongue was present above immature granulation tissue. Although deeper layers showed signs of granulation tissue maturation, inflammatory cells were still visible, and the overall architecture appeared less mature compared to the silver sulfadiazine group (black arrowhead). The fennel-treated group (Fig. 3 D, D 1) exhibited the most advanced healing features. A fully re-epithelialized surface with a well-organized, collagen-rich granulation tissue was observed. Inflammatory cell presence was minimal, and both blood capillaries and oedema were markedly reduced (white arrowhead), reflecting significant maturation and remodelling of the wound bed compared to purslane group.

Discussion

Second-degree burns involve injury to both the outermost skin layer (epidermis) and the layer beneath it (dermis), resulting in pain, blisters, and potential scarring. Properly caring for these wounds, maintaining moisture levels, and reducing inflammation are crucial for healing. While conventional treatments like ointments and bandages are commonly employed, natural remedies such as fennel (*Foeniculum vulgare*) and purslane (*Portulaca oleracea*) are garnering interest for their potential healing benefits. Fennel's anti-inflammatory, antimicrobial, and antioxidant characteristics may support tissue repair, while purslane's omega-3 fatty acids and antioxidants could accelerate healing, alleviate pain, and minimize scarring [17, 18].

Regarding the wound area, from day 15 to 21 after the burn, the purslane and fennel groups showed better wound closure compared to the control group and the group treated with 1% silver sulphadiazine. Furthermore, the fennel group demonstrated a more significant improvement in wound closure when compared to the purslane group.

Animal studies have provided additional evidence to support these findings. In a study conducted by Kumar et al. the researchers reported that the topical application of *P. oleracea* extract in rat models significantly accelerated the process of wound closure, enhanced the deposition of collagen, and promoted the formation of new epithelial tissue [19].

The positive effects of *P. oleracea* are particularly beneficial for chronic wounds, especially in individuals with diabetes. Lee et al. found that diabetic mice treated with *P. oleracea* extract showed elevated levels of vascular endothelial growth factor (VEGF), which promoted the formation of new blood vessels. Furthermore, the extract was able to stimulate fibroblast activity and collagen production, processes that are frequently disrupted in the wound healing of diabetic patients [20].

Collectively, these findings reinforce the therapeutic value of *P. oleracea* in wound healing by modulating inflammation, promoting collagen synthesis and angiogenesis, and exhibiting antimicrobial effects.

Several studies have highlighted the wound-healing potential of *Foeniculum vulgare* through its diverse pharmacological properties. Sahane et al. demonstrated that topical application of a 7% fennel ointment significantly enhanced wound contraction and reduced wound area over a 16-day period, confirming its role in tissue regeneration [21].

Expanding on these findings, Keskin, I., et al. [22] investigated the effects of fennel extract in rat models with incisional and excisional wounds. Their results indicated a marked reduction in wound size, increased granulation tissue formation, and elevated hydroxyproline levels, which are indicative of enhanced collagen synthesis.

In this study, purslane and fennel incorporated into hydrogel formulations accelerated burn wound healing by enhancing collagen synthesis, epithelialization, and angiogenesis. Purslane was effective in promoting rapid tissue regeneration, while fennel exhibited superior wound closure outcomes due to its anti-inflammatory, antioxidant, and antimicrobial properties. By day 21, both botanical treatments significantly outperformed the control and silver sulfadiazine groups, with fennel showing the most pronounced improvement.

The histological outcomes observed in this study align with and extend the current body of research regarding the therapeutic efficacy of natural plant extracts and conventional treatments in burn wound healing.

In the control group, delayed healing characterized by necrotic scab formation, epithelial loss, and immature granulation tissue mirrors findings reported by Tavares Pereira et al. who demonstrated that untreated deep second-degree burns exhibit persistent inflammation, extensive necrosis, and poor tissue organization up to three weeks post-injury [15].

Treatment with silver sulfadiazine (SSD) led to significant improvements, including marked re-epithelialization and vascularized granulation tissue. These findings are consistent with Atiyeh et al., who highlighted SSD's antimicrobial properties and its capacity to reduce infection-related delays in wound healing [23]. However, our findings also echo concerns raised in previous studies indicating that SSD, despite controlling microbial burden, may delay fibroblast activity and collagen remodeling due to cytotoxic effects on host cells [24].

The purslane-treated group displayed moderate wound healing, with partial re-epithelialization and maturing granulation tissue. This result is supported by Zarei et al., who demonstrated that topical application of Portulaca oleracea extract on excisional wounds in rats accelerated epithelial formation and reduced oxidative stress markers [25]. However, as also noted in our study, the incomplete resolution of inflammation suggests that while purslane has beneficial bioactive compounds (e.g., omega-3 fatty acids, flavonoids), its therapeutic effects may be slower or less potent in deep burns compared to conventional agents.

The most striking results were observed in the fennel-treated group, which showed advanced granulation tissue maturation, dense collagen deposition, and minimal inflammatory infiltration by day 21. This supports findings by Rather et al. who reported that Foeniculum vulgare possesses strong anti-inflammatory and antioxidant activities, promoting fibroblast proliferation and ECM synthesis [26]. Furthermore, Kishore et al. (2018) demonstrated that fennel extract significantly enhanced wound closure and increased hydroxyproline content (a marker of collagen) in animal models, further corroborating our histological observations [20].

Compared to these prior studies, the current investigation confirms the potential of fennel as a highly effective alternative to SSD, especially in promoting late-stage healing and tissue remodeling. While SSD showed efficient early-phase wound control, fennel demonstrated more comprehensive regenerative effects, suggesting a potential synergistic or sequential use in therapeutic applications.

Conclusion

The findings underscore the therapeutic promise of *Foeniculum vulgare* and *Portulaca oleracea* in the treatment of second-degree burns. Notably, F. vulgare demonstrated greater efficacy, suggesting its superior potential as a natural alternative or complementary agent in wound care. Its pronounced pharmacological effects make it especially valuable for accelerating healing and reducing inflammation. While P. oleracea also showed beneficial properties, F. vulgare may offer more consistent therapeutic outcomes. Further clinical studies are warranted to refine the formulations, dosage, and application protocols, particularly for F. vulgare, to advance its use in human burn management.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the ethics guidelines of the Faculty of Veterinary Medicine, Kafr Elsheikh University, Egypt (ethics approval number; KFS-IACUC/267/2025).

Fig. 1. The influence of different treatments in burn wound healing

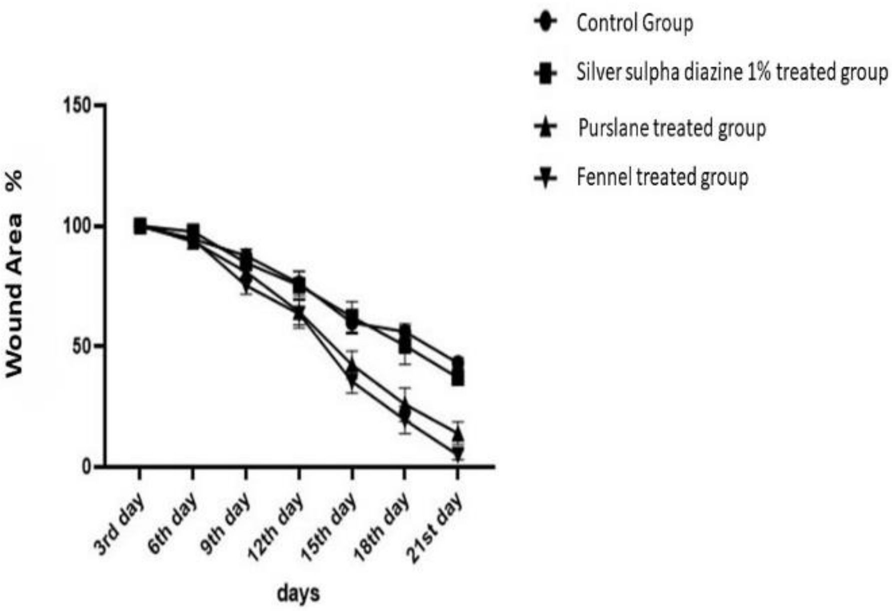

Fig. 2. Effect of purslane and fennel on wound closure in rats.
The wound closure rate was expressed as the percent of wound healing area compared with that on post-burn day 3 (zero)

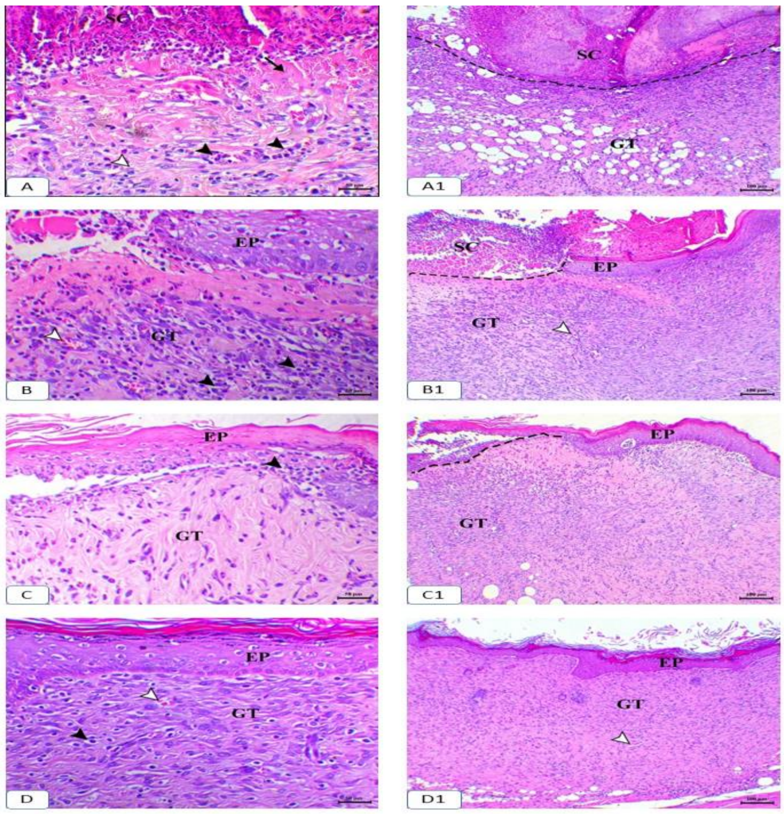

Fig. 3. Histological evaluation of burn wound healing in different treatment groups at day 21 post-burn (H&E staining).(A, A 1): Control non-treated group showing thick necrotic scab (SC), complete epithelial loss, and immature granulation tissue (GT) with widespread inflammatory cells (black arrowheads).
(B, B 1): Silver sulfadiazine-treated group showing re-epithelialization (EP), organized GT with perpendicular capillaries and inflammatory infiltration (white arrowhead), and reduced SC.
(C, C 1): Purslane-treated group exhibiting epithelial tongue (EP), immature GT with signs of maturation and remaining inflammatory cells (black arrowhead).
(D, D 1): Fennel-treated group displaying complete re-epithelialization, dense collagen-rich GT, and minimal inflammatory infiltration (white arrowhead). Scale bars = 50 μm.

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دراسه تأثير خلاصة الشمر والرجله على التئام الجروح بسبب الحروق في الفئران

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الملخص

تمثل الحروق تحديًا كبيرًا في الرعاية السريرية، ولا يزال البحث عن علاجات فعّالة أولوية ملحة. تهدف هذه الدراسة إلى استكشاف القدرة العلاجية لخلاصة نباتي الشمر والرجله في نموذج تجريبي لحروق الجلد العميقة من الدرجة الثانية باستخدام الفئران. تم استخدام تركيبات هلامية (هيدروچيل) تحتوي على خلاصة الشمر والرجله على مواضع الحروق، وتم تقييم تأثيرها على إغلاق الجروح. أظهرت النتائج أن كلا من علاجي الشمر والرجله ساهما بشكل ملحوظ في تسريع التئام الجروح. فقد أظهرت الجروح المعالجة بالشمر إعادة التغطية الظهارية بشكل كامل، وترسيبًا منظمًا لألياف الكولاجين، والتهابًا طفيفًا، في حين أظهرت الجروح المعالجة بالرجله تحسنًا متوسطًا مع تجدد جزئي للظهارة. أما العلاج بسلفاديازين الفضة فقد أدى إلى إعادة التغطية الظهارية ولكنه لم يعزز نضوج الأنسجة بنفس الدرجة. تشير هذه النتائج إلى أن الشمر والرجله يمثلان بدائل طبيعية واعدة في علاج الحروق، مع تفوق واضح للشمر في تعزيز التئام الجروح وتجديد الأنسجة.

الكلمات الدالة: حروق، الرجله، الشمر، الهيدروچيل، الالتهاب.