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Histopathological and Biochemical Evaluation of the Effect of *Calendula officinalis* Extract on Skin Wound Healing in Rats

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This study aimed to investigate the histopathological and biochemical effects of *Calendula officinalis* (*C. officinalis*) on cutaneous wound healing in rats. Twenty-four *Wistar albino* rats were were randomly allocated into three groups (n = 8 each): Control, *C. asiatica*, and *C. officinalis*. Circular excision wounds (8 mm in diameter) were created in the interscapular region using a biopsy punch under xylazine-ketamine anesthesia. *C. asiatica* and *C. officinalis* ointments were applied topically once daily to their respective treatment groups, while glycerin ointment was administered to the Control group. Blood and skin tissue samples were collected on post-injury days 7 and 15 for histopathological and biochemical analyses. Both *C. asiatica* and *C. officinalis* groups demonstrated significantly greater wound contraction compared to the Control group. Histopathological evaluation revealed that topical application of *C. officinalis* exerted anti-inflammatory effects by reducing inflammation and edema, while promoting epithelialization, fibroblast proliferation, and collagen deposition. Biochemical analyses showed significant reduction in malondialdehyde levels, restoration of antioxidant defense system components, and improvement in several biochemical parameters in the *C. officinalis* group. Topical application of *C. officinalis* significantly accelerated wound healing by shortening epithelialization time, enhancing connective tissue proliferation, and restoring oxidative balance.

Key Words: *Calendula officinalis, skin, wound healing, rat*

Ratlarda *Calendula officinalis* Ekstraktının Derideki Yara İyileşmesi Üzerine Etkisinin Histopatolojik ve Biyokimyasal Olarak Değerlendirilmesi

Bu çalışmanın amacı, *Calendula officinalis*'in ratlarda deri yara iyileşmesi üzerine etkilerini histopatolojik ve biyokimyasal olarak araştırmaktır. Yirmi dört adet Wistar albino sıçan rastgele üç gruba ayrıldı (n = 8): Kontrol, *C. asiatica* ve *C. officinalis*. Ksilazin-ketamin anestezisi altında biyopsi punch kullanılarak interskapular bölgeye 8 mm çapında dairesel eksizyon yaraları oluşturulmuştur. İlgili tedavi gruplarına *C. asiatica* ve *C. officinalis* merhemleri günde bir kez topikal olarak uygulanırken, kontrol grubuna gliserin merhemi uygulanmıştır. Yara oluşumundan sonraki 7. ve 15. günlerde kan ve deri doku örnekleri histopatolojik ve biyokimyasal analizler için alınmıştır. Hem *C. asiatica* hem de *C. officinalis* gruplarında, kontrol grubuna kıyasla anlamlı derecede daha yüksek yara kontraksiyonu gözlenmiştir. Histopatolojik değerlendirme, *C. officinalis*'in topikal uygulanmasının inflamasyonu ve ödemi azaltarak antiinflamatuar etki gösterdiğini; epitelizasyonu, fibroblast proliferasyonunu ve kollajen birikimini desteklediğini ortaya koymuştur. Biyokimyasal analizler ise malondialdehit düzeylerinin normalleştiğini, antioksidan savunma sistemi bileşenlerinin yeniden düzenlendiğini ve çeşitli biyokimyasal parametrelerde iyileşme sağlandığını göstermiştir. Topikal *C. officinalis* uygulaması, epitelizasyon süresini kısaltarak, bağ dokusu proliferasyonunu artırarak ve oksidatif dengeyi yeniden sağlayarak yara iyileşmesini anlamlı düzeyde hızlandırmıştır.

Anahtar Kelimeler: *Calendula officinalis, deri, yara iyileşmesi, rat*

Introduction

A wound is defined as a disruption of the normal anatomical structure and, its physiological function (1). Proper wound healing is essential to restore both the structural integrity and physiological capacity of the skin. Wound repair is a complex and tightly regulated biological process that restores damaged tissues through a series of overlapping phases: hemostasis, inflammation, proliferation (granulation tissue formation), and remodeling (scar formation) (2, 3).

During the inflammatory phase, polymorphonuclear neutrophils (PMNs) and macrophages infiltrate the wound site to eliminate debris and pathogens. Angiogenesis simultaneously ensures nutrient and oxygen supply to regenerating cells (4, 5). In the proliferative phase, fibroblasts form granulation tissue composed of a provisional extracellular matrix rich in collagen, fibronectin, and hyaluronic acid (6, 7). This stage plays a pivotal role in re-establishing structural stability, while the remodeling phase strengthens the extracellular matrix and restores functional integrity (8).

Historically, medicinal plants and herbal preparations have been widely used in wound care, often based on empirical knowledge rather than validated scientific evidence (9, 10). Despite this, herbal agents continue to attract attention due to their accessibility, affordability, and potential clinical efficacy (11).

C. officinalis (marigold) has traditionally been employed in Mediterranean cultures for the treatment of skin lesions (12). Its wound-healing potential is attributed primarily to anti-inflammatory activity. Phytochemical studies have identified a wide variety of active compounds, including flavonoids, coumarins, steroids, terpenoids, carbohydrates, lipids, tocopherols, quinones, carotenoids, essential oils, fatty acids, and minerals (13-15).

Based on these properties, the present study aimed to investigate the histopathological and biochemical effects of *C. officinalis* on wound healing and epithelialization in a rat model.

Materials and Methods

Research and Publication Ethics: The study protocol received approval from the Ethics Committee for Experimental Animals at Yüzüncü Yıl University (2015/10), and all methods were conducted in compliance with internationally accepted guidelines for the care and use of laboratory animals.

Laboratory Animals: Twenty-four male *Wistar albino* rats (200–240 g) were obtained from the Experimental Animal Center of Yüzüncü Yıl University. Animals were housed under standard laboratory conditions [25±2°C, relative humidity (50±15%), 12-h light/dark cycle] in individual wire-bottom cages with free access to food and water. Veterinary supervision was maintained throughout the study.

Plant Material and Extract Preparation: *C. officinalis* flowers were purchased from a local herbal store in Van, Turkey. The flowers were shade-dried, ground into powder, and 100 g of the material was extracted with 96% (v/v) ethanol using a Soxhlet apparatus. The extract was concentrated under reduced pressure at 40–45 °C using a rotary evaporator. A wound-healing ointment containing 5% (w/w) *C. officinalis* extract in glycerin was prepared and stored in amber-colored glass bottles until use.

Experimental Design and Wound Model: Rats were randomly divided into three groups (n = 8 per group):

Group 1 (Control): Received glycerin ointment only,

Group 2 (Reference-*Centella asiatica*): Treated with Madecassol® ointment.

Group 3 (*C. officinalis*): Treated with *C. officinalis* ointment.

To evaluate the wound healing potential of C. a officinalis extract, a topical pomade containing 1% Centella asiatica extract (Madecassol®; Bayer Türk Kimya San. Ltd. Şti., İstanbul, Türkiye) was employed as the positive control group. This topical preparation was utilized as a reference standard in the study due to the well-documented efficacy of C. asiatica on collagen synthesis and epithelialization.

Anesthesia was induced via intraperitoneal injection of ketamine hydrochloride (50 mg/kg body weight) and xylazine hydrochloride (10 mg/kg body weight). The dorsal region was shaved, depilated, and disinfected with antiseptic. A full-thickness circular excision wound (8 mm diameter) was created in the interscapular region using a sterile biopsy punch, extending into the subcutaneous tissue (Figure 1).

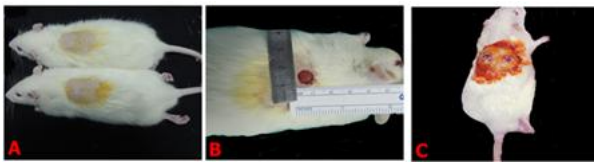

Figure 1. Experimental design and procedure. **A.** Skin surface prior to wound creation. **B.** Postoperative topical treatment application. **C.** Measurement of wound area.

Treatments were applied topically once daily at 24-hour intervals, beginning immediately after wound induction and continuing until complete healing. Wound areas were measured on days 0, 4, 7, 12, and 15 by tracing margins onto millimeter graph paper, with surface areas calculated using AutoCAD software. Standardized digital photographs were taken at a fixed distance of 15 cm perpendicular to the wound surface. The animals were monitored clinically daily.

On days 7 and 15, four rats from each group were euthanized under anesthesia. Blood and wound tissues were collected for histopathological and biochemical analyses.

Histopathological Examination: Full-thickness wound tissues were excised, fixed in 10% neutral-buffered formalin for 48 h, dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin. Serial sections of 5 µm were prepared using a microtome and stained with hematoxylin and eosin (H&E). Sections were examined under light microscopy, and photomicrographs were obtained.

Histopathological parameters were semi-quantitatively scored as absent (-), mild (+), moderate (++), or severe (+++) for epidermal and dermal remodeling. Parameters included re-epithelialization, ulceration, fibroblast proliferation, mononuclear/polymorphonuclear cell infiltration, neovascularization, and collagen deposition. Based on these parameters, wounds were classified into inflammatory, proliferative, or remodeling phases (16).

Biochemical Analysis

Lipid peroxidation: Malondialdehyde (MDA) concentrations were determined according to Jain et al. (17) based on thiobarbituric acid reactivity.

Antioxidant substances and enzymes: Glutathione (GSH) levels were measured by the method of Beutler et al. (18). Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined spectrophotometrically at 505 nm and 37 °C based on inhibition of formazan dye formation (19). Catalase (CAT, EC 1.11.1.6) activity was determined using the method of Aebi (20).

Serum biochemistry: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total protein (TP), creatine kinase (CK), iron (Fe), glucose (GLU), albumin (ALB), phosphorus (P), magnesium (Mg), calcium (Ca), and urea were measured using an automated analyzer (BM/HITACHI-911) with commercial kits (DPC; Diagnostic Products Corporation, USA).

Statistical Analysis: All data were presented as means ± standard deviations. The statistical analyses were performed using the Minitab 13 for Windows. Normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene's tests, respectively. Differences among groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. A p value ≤0.05 was considered statistically significant. The Kruskal–Wallis test was used as a nonparametric alternative to evaluate histopathological scores.

Results

Wound Healing Activity: Wound area analysis demonstrated that baseline measurements on day 1 were comparable across all groups, with no statistically significant differences (p>0.05). However, as healing progressed, significant intergroup variations emerged. On day 4, wound areas were significantly reduced in both the *C. asiatica* and *C. officinalis* groups compared with controls (p≤0.05), reflecting an early therapeutic benefit. By day 7, wound contraction had accelerated in both treatment groups, yielding similar healing patterns; nevertheless, both remained significantly different from the control (p≤0.05). On day 12, wound closure continued to advance more effectively in the treatment groups (p≤0.05). By day 15, wound areas were markedly smaller in both the *C. asiatica* and *C. officinalis* groups than in the control (p≤0.05), confirming their long-term efficacy. Collectively, these findings suggest that both interventions promoted wound repair throughout the study period. Notably, *C. asiatica* produced earlier benefits on days 4 and 7, although both treatments consistently outperformed the control. When the *C. asiatica* group was compared with the *C. officinalis* group, a statistically significant difference was observed only on day 12. Wound healing was faster in the *C. asiatica* group than in the *C. officinalis* (p≤0.05). On the other measurement days, no statistically significant differences were found between the two groups (p>0.05). The effects of *C. officinalis* on wound closure are depicted in Figure 2.

Macroscopic Findings: Macroscopic evaluation on days 0, 4, 7, 12, and 15 revealed distinct differences in wound morphology among groups. On day 4, control wounds exhibited irregular scab formation with heterogeneous crusting and localized exudation, whereas both treatment groups showed compact, homogeneous scabs. By day 7, control wounds still demonstrated irregular scabbing and persistent inflammation, while treatment groups displayed organized wound edges and accelerated epithelialization. On day 12, healing in controls was delayed, with incomplete scab loss and persistent fissures, whereas treatment groups showed advanced contraction, granulation tissue replacement, and near-complete scab removal. By day 15, residual scabs and scarring persisted in controls, while both treatment groups demonstrated nearly complete closure, minimal scarring, and uniform epithelial renewal. Representative macroscopic images are shown in Figure 3 (A–F).

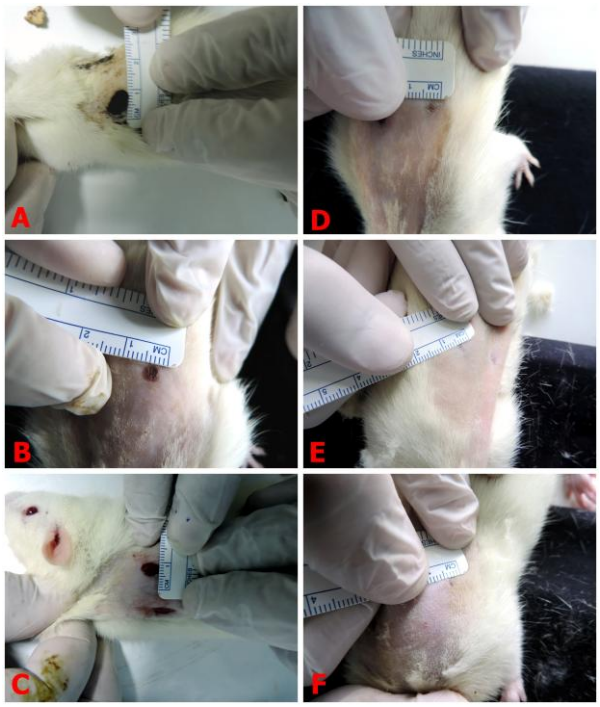

Figure 2. Representative macroscopic images of wound healing in the Control, *C. asiatica*, and *C. officinalis* groups on days 7 and 15. (A–C) Day 7; (D–F) Day 15

Table 1. Histopathological evaluation of wound healing parameters in experimental groups

Groups	Wound healing processes							Healing phases		
	S	Re	FP	Cd	MNC	PMN	Nv	I	P	R
Control	+++	-	+	-/+	++/+++	+++	-/+	+/++	+/++	+/++
C. asiatica	+/++	+/++	++	++	+	++	+/++	-/+	++	++/+++
C. officinalis	+/++	+	++	++	+	++	++	-/+	+	++/+++

H&E-stained sections were scored as negative (-), mild (+), moderate (++) and severe (+++) for epidermal and/or dermal re-modeling. S: Scab, Re: Re-epithelization, FP: Fibroblast proliferation, Cd: Collagen deposition, MNC: Mononuclear cells, PMN: Polymorphonuclear cells, Nv: Neovascularization, I: Inflammatory phase, P: Proliferation phase, R: Re-modeling phase

Microscopic Findings: Figure 4 presents the microscopic findings according to the groups on the 7 (Figure 4 A-C) and 15 (Figure 4 D-F) days for wound healing process and healing phases. Histopathological analysis revealed clear differences in wound healing progression. In the control group, extensive scab formation (+++), moderate ulceration (++), absent or minimal re-epithelialization (−/+), weak fibroblast proliferation (+), irregular collagen deposition (−/+), and marked inflammatory cell infiltration (MNC ++/+++, PMN +++) were noted. Neovascularization was limited (−/+), and the healing phases remained insufficient (Figure 4 A and D). In contrast, *C. asiatica* treatment resulted in limited scabbing (++), absence of ulceration (−), advanced re-epithelialization (+/++), pronounced fibroblast proliferation (++), and organized collagen deposition (++). Inflammatory infiltration was reduced, neovascularization was moderate (+/++), and proliferative/remodeling phases were prominent (++/+++) (Figure 4 B and E). The *C. officinalis* group showed a comparable profile: reduced scabbing (++), minimal ulceration (−/+), advanced re-epithelialization (+), marked fibroblast proliferation (++), regular collagen deposition (++), low inflammatory infiltration, and enhanced neovascularization (++). Both treatments facilitated efficient progression through healing phases, contrasting with the inflammation-dominant control group (Figure 4C and F). Quantitative histopathological scores are presented in Table 1.

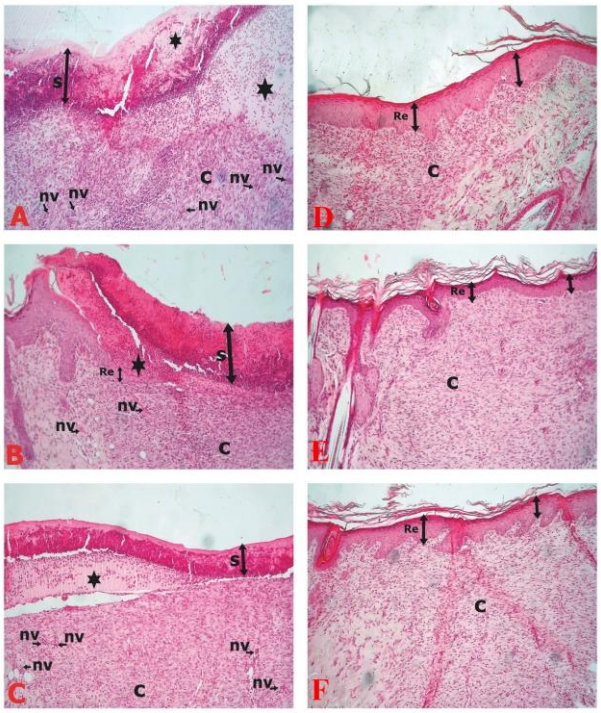

Figure 3. Microscopic appearance of wound healing on days 7 and 15. H&E. **A.** Control group, day 7. **B.** *C. asiatica* group, day 7. **C.** *C. officinalis* group, day 7. **D.** Control group, day 15. **E.** *C. asiatica* group, day 15. **F.** *C. officinalis* group, day 15. Abbreviations: S: Scab, Re: Re-epithelization, nv: neovascularization, stars: exudate, c: connective tissue

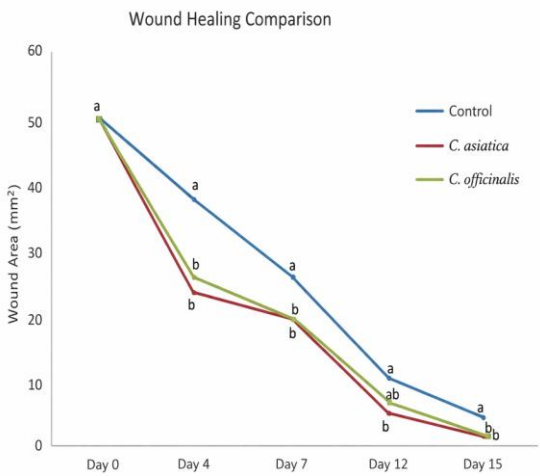

Figure 4. Histopathological evaluation of wound healing and epidermal/dermal remodeling in control, *C. asiatica*, and *C. officinalis*-treated rats on days 7 and 15. Hematoxylin and eosin (H&E) staining. **A.**Control group, day 7. **B.** *C. asiatica* group, day 7. **C.** *C. officinalis* group, day 7. **D.** Control group, day 15. **E.** *C. asiatica* group, day 15. **F.** *C. officinalis* group, day 15.

Lipid Peroxidation and Antioxidant Parameters: Control group wounds exhibited significantly elevated malondialdehyde (MDA) levels. Both *C. asiatica* and *C. officinalis* significantly reduced MDA concentrations ($p \leq 0.05$). Endogenous antioxidant defense markers were also improved: glutathione (GSH) levels, diminished in controls, were significantly increased in both treatment groups; superoxide dismutase (SOD) activity was higher, most prominently in the *C. asiatica* group; and catalase (CAT) activity, reduced in controls, was significantly elevated in the treatment groups, with a more robust effect in *C. asiatica*. These results indicate that both treatments attenuated lipid peroxidation and enhanced antioxidant defense, contributing to accelerated repair. Data are summarized in Table 2.

Biochemical Findings: Biochemical assessment revealed significant group differences, particularly in hepatic enzyme activity and protein metabolism. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were elevated in controls but significantly reduced in both treatment groups ($p \leq 0.05$). Gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), urea, calcium, magnesium, phosphorus, creatine kinase (CK), glucose, and iron levels showed no significant variation ($p > 0.05$). Total protein and albumin levels were significantly increased in treatment groups ($p \leq 0.05$), reflecting improved protein synthesis. Collectively, these results suggest hepatoprotective effects and improved protein homeostasis by both interventions, without adverse effects on glucose or mineral balance. Biochemical data are summarized in Table 3.

Table 2. The effect of *C. officinalis* on MDA, GSH contents and antioxidant enzyme activities in wound healing tissue

Parameters	Control X± SD	*C. asiatica* X± SD	*C. officinalis* X± SD
MDA (nmol/g)	161.24±15.32[a]	102.67±3.47[b]	126.32±16.42[b]
GSH (mg/g)	2.31±1.04[a]	3.87±0.42[b]	3.59±0.72[b]
SOD (U/L)	2016.18±23.51[a]	2372.20±21.13[b]	2213.62±18.41[b]
CAT (U/L)	58.34±7.32[a]	87.12±4.62[b]	82.43±6.21[b]

Data are expressed as mean ± SD. Different superscript letters indicate significant differences ($p \leq 0.05$). MDA: Malondialdehyde; GSH: Reduced glutathione; SOD: Superoxide dismutase; CAT: Catalase

Table 3. The effect of *C. officinalis* on some biochemical parameters in wound healing model in rats

Parameters	Control X± SD	*C. asiatica* X± SD	*C. officinalis* X± SD
ALT (U/L)	123.21±5.10[a]	83.4±2.18[b]	89.16±3.15[b]
AST (U/L)	208±16.2[a]	152±17.23[b]	159.30±19.16[b]
GGT (U/L)	0.41±0.15[a]	0.39±0.57[a]	0.40±0.12[a]
ALP (U/L)	512±21.42[a]	478±23.14[a]	488.13±12.35[a]
UREA (mg/dL)	53.14±8.15[a]	48.24±3.2[a]	49.47±3.11[a]
Ca (mg/dL)	10.23±0.14[a]	10.73±0.19[a]	11.13±0.17[a]
Mg (mg/dL)	3.76±0.14[a]	4.23±0.41[a]	4.12±0.13[a]
P (mg/dL)	4.53±0.21[a]	4.97±0.18[a]	4.52±0.14[a]
TP (g/dL)	6.53±0.54[a]	8.96±0.12[b]	8.23±0.21[b]
ALB (g/dL)	3.73±0.54[a]	4.87±0.53[b]	4.76±0.12[b]
CK (U/L)	1109.28±21.4[a]	953.16±12.11[a]	994.19±20.12[a]
GLU (mg/dL)	104.41±1.21[a]	89.42±3.31[a]	94.02±1.12[a]
Fe (ng/mL)	54.32±1.14[a]	58.13±1.28[a]	56.18±1.23[a]

Data were expressed as mean±SD. Different superscript letters indicate significant differences ($p \leq 0.05$).

Discussion

Dermal wound healing represents a highly coordinated and dynamic biological process essential for restoring the structural and functional integrity of the skin (21). This process proceeds through a series of overlapping phases—hemostasis, inflammation, proliferation, and remodeling—involving complex interactions among keratinocytes, fibroblasts, endothelial and inflammatory cells, as well as extracellular matrix components (3, 7). Although the skin possesses inherent regenerative capacity, the use of therapeutic agents can markedly enhance and accelerate these natural repair mechanisms (22). Plants have played a central role in healthcare since antiquity, serving as primary sources of both nutrition and therapeutic agents. In this regard, medicinal plants such as *C. officinalis* have attracted considerable interest as accessible, cost-effective, and safe alternatives. According to the World Health Organization, approximately 80% of the global population relies on plant-derived remedies for primary healthcare needs (23). Numerous studies support the efficacy of plant-derived compounds in wound healing, citing their widespread availability, minimal side effects, and pharmacological properties (24). This study investigated the wound-healing potential of topical *C. officinalis* in a rat full-thickness excisional wound model.

In the present study, both *C. asiatica* and *C.officinalis* significantly enhanced wound contraction and closure compared with the control group, with measurable benefits evident from day 4 onward. Notably, *C. asiatica* demonstrated slightly earlier effects, whereas *C. officinalis* exhibited sustained healing throughout the observation period. The observed improvements are likely mediated by the plant's bioactive constituents, including flavonoids, triterpenes, and saponins, which exert antimicrobial, anti-inflammatory, and antioxidant effects (12). Our findings are consistent with previous studies demonstrating the efficacy of *C. officinalis* and other phytotherapeutic agents. Ozturan and Akin (25) reported that *C. officinalis* extract shortened epithelialization time, reduced inflammation, and promoted fibroblast proliferation and collagen synthesis. Similarly, Parente et al. (26) demonstrated that *C. officinalis* positively influenced the proliferative phase and wound closure, likely due to its antimicrobial and reparative properties. In our study, by day 12, wounds in both treatment groups showed substantial closure, while those in the control group remained larger. By day 15, wounds in the treatment groups were nearly completely healed, consistent with the findings of Ejiohuo et al. (12) and Preethi and Kuttan (27), who attributed the plant's wound-healing activity to bioactive compounds such as flavonoids, triterpenes, and saponins that mitigate oxidative stress by scavenging free radicals. These mechanisms collectively suggest that *C. officinalis* accelerates wound healing by modulating fibroblast function, extracellular matrix deposition, and angiogenesis. Taken together, *C.officinalis* demonstrated wound-healing effects comparable to *C. asiatica*, significantly reducing wound

size and promoting tissue regeneration. While *C. asiatica* remains a well-established pharmaceutical standard, *C. officinalis* exhibited comparable efficacy despite being a botanical preparation, supporting its potential use as a complementary or alternative therapy for wound management.

The cellular and extracellular matrix dynamics within the epidermal and dermal layers are influenced by both local and systemic factors. In the present study, topical application of *C. asiatica* and *C. officinalis* extract led to marked histopathological improvements compared with the control group, reflecting their beneficial effects across multiple phases of tissue repair. Scab formation was notably more evident in controls but reduced in treated groups, indicating accelerated healing and earlier resolution of the wound surface (3). Consistent with earlier findings, such rapid scab detachment corresponds to enhanced re-epithelialization and fibroblast proliferation (28, 29). Ulceration, which was moderate in the control animals, was absent or minimal in treated groups, supporting more effective epithelial restoration. Re-epithelialization was limited in the control group but well advanced in both *C. asiatica* and *C. officinalis*-treated rats, confirming their stimulatory roles in epidermal regeneration. These outcomes align with previous evidence that *C. asiatica* triterpenoids enhance fibroblast activity and collagen synthesis (30, 31), while flavonoids in *C. officinalis* facilitate epithelialization and wound contraction (26). Accordingly, fibroblast proliferation and collagen deposition were significantly greater in the treatment groups, indicating enhanced extracellular matrix remodeling (32). Inflammatory cell infiltration, which was intense in the control wounds, was markedly reduced in the treated groups, consistent with the documented anti-inflammatory properties of both *C. asiatica* and *C. officinalis* (27, 33). Furthermore, angiogenesis was substantially increased following treatment, ensuring better vascularization of the healing tissue and supporting previous reports of the pro-angiogenic activity of *C. officinalis* (34). Overall, these findings demonstrate that both *C. asiatica* and *C. officinalis* significantly enhance wound repair by promoting re-epithelialization, fibroblast proliferation, collagen synthesis, and angiogenesis while attenuating inflammatory responses.

Oxidative stress is a major determinant of wound healing, as excessive reactive oxygen species (ROS) impair cellular proliferation, angiogenesis, and extracellular matrix remodeling, ultimately delaying tissue repair (35). In this study, significant intergroup differences were observed in both lipid peroxidation and antioxidant parameters, emphasizing the importance of redox balance in cutaneous regeneration. MDA, a key marker of lipid peroxidation and oxidative injury, was markedly elevated in controls, indicating increased oxidative stress and delayed healing. Conversely, *C. asiatica* and *C. officinalis* treatments significantly reduced MDA levels, consistent with previous reports on their antioxidative and wound-healing activities (26). GSH, a critical intracellular antioxidant maintaining redox homeostasis, was significantly elevated in both treatment groups, suggesting enhanced endogenous antioxidant capacity. Similar upregulation of GSH has been linked to triterpenoid components of *C. asiatica* and phenolic compounds in *C. officinalis* (27). SOD, responsible for converting superoxide radicals into less reactive species (35), exhibited higher activity in *C. asiatica* treated rats and moderate elevation in the *C. officinalis* group, supporting an improved oxidative environment favorable for tissue repair (36). CAT, which decomposes hydrogen peroxide into water and oxygen, followed a similar trend, with maximal activity in *C. asiatica*, intermediate in *C. officinalis*, and minimal in controls. Enhanced SOD and CAT activity reflects strengthened enzymatic defenses and reduced oxidative burden, thereby facilitating re-epithelialization and collagen synthesis (33). Collectively, these findings demonstrate that both *C. asiatica* and *C. officinalis* exert potent antioxidant effects by decreasing lipid peroxidation and enhancing enzymatic (SOD, CAT) and non-enzymatic (GSH) defenses. Although *C. asiatica* showed slightly stronger activity, *C. officinalis* exhibited robust antioxidant potential, validating its role as an effective phytotherapeutic adjunct in wound management. The biochemical improvements observed likely underlie the histopathological outcomes, linking enhanced oxidative balance to accelerated tissue regeneration.

In this study, systemic biochemical parameters were analyzed to evaluate the effects of *C. asiatica* and *C. officinalis* on experimentally induced skin wounds. Significant group differences were observed in ALT, AST, total protein, albumin, while GGT, ALP, urea, calcium, magnesium, phosphorus, CK, glucose, and iron showed no marked variation. The reductions in ALT and AST levels in both treatment groups suggest attenuation of hepatic enzyme activity, indicative of reduced hepatocellular stress. This hepatoprotective effect may result from decreased oxidative damage, consistent with previous findings that antioxidant therapies normalize elevated liver enzyme levels following tissue injury (7, 37). The improvement observed in the *C. officinalis* group likely stems from its high flavonoid content and free radical–scavenging capacity (27). Increases in total protein and albumin levels further indicate enhanced protein synthesis and improved metabolic support for wound repair. As albumin contributes to cellular proliferation, collagen formation, and tissue remodeling (28), these changes reflect stimulation of regenerative processes, aligning with the reported immunomodulatory and reparative actions of *C. officinalis* (26). No significant differences were noted in other biochemical markers, indicating systemic stability during the healing process. Collectively, these findings demonstrate that both *C. asiatica* and *C. officinalis* modulate hepatic enzyme activity, enhance protein metabolism, and protect against tissue damage, thereby supporting both local and systemic aspects of wound healing.

In conclusion, *C. officinalis* exhibited significant wound-healing potential by promoting cellular proliferation, enhancing extracellular matrix formation, and facilitating organized tissue remodeling. Moreover, it improved key biochemical and histological parameters

by mitigating oxidative stress and restoring redox balance, thereby creating a microenvironment conducive to effective tissue repair. These findings provide strong scientific support for its traditional medicinal application and underscore its promise as a safe, accessible, and effective phytotherapeutic agent for enhancing cutaneous wound healing in clinical settings.

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