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Effectiveness of a Phytotherapeutic Cream Formulated with *Juglans regia, Argania spinosa, Prunus dulcis,* and *Lavandula angustifolia* in the Treatment of Second-Degree Burns: A Biochemical and Histopathological Study

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Abstract: Burn injuries are complex pathological conditions that necessitate effective therapeutic strategies. This study investigated the biochemical and histopathological effects of a phytotherapeutic cream composed of *Juglans regia* leaf extract, *Argania spinosa* kernel oil, *Prunus dulcis* oil, and *Lavandula angustifolia* oil on second-degree burn wounds in male Wistar rats (n = 48). Animals were randomly assigned to four groups: Healthy Control, Burn Control, Silver Sulfadiazine (1% cream), and JAPL (herbal) cream. Treatments were topically applied once daily for 21 days. Biochemical analysis revealed a statistically significant increase in superoxide dismutase (SOD) levels in the JAPL group on day 21 (89.3 ± 4.6 ng/mL; $p < 0.001$) and a concomitant reduction in malondialdehyde (MDA) levels (142.5 ± 7.2 nmol/L; $p < 0.01$), indicating enhanced antioxidant activity and reduced lipid peroxidation. Histopathological evaluation demonstrated epithelial regeneration, fibroblast proliferation, and improved keratinization by day 21 in the JAPL group, surpassing the effects observed with silver sulfadiazine. These findings suggest that the tested herbal formulation may serve as a potential alternative for managing second-degree burns, though further clinical research is warranted to confirm its efficacy in humans.

Keywords: Antioxidant defense, *Argania spinose*, histopathology, *Juglans regia*, phytotherapy, second-degree burns.

Juglans regia, Argania spinosa, Prunus dulcis ve Lavandula angustifolia ile Formüle Edilmiş Bir Fitoterapötik Kremin İkinci Derece Yanıkların Tedavisinde Etkinliği: Biyokimyasal ve Histopatolojik Bir Çalışma

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Öz: Yanık yaralanmaları, etkili terapötik stratejiler gerektiren karmaşık patolojik durumlardır. Bu çalışmada, *Juglans regia* yaprak ekstresi, *Argania spinosa* çekirdek yağı, *Prunus dulcis* yağı ve *Lavandula angustifolia* yağı içeren fitoterapötik bir krem formülasyonunun ikinci derece yanık yaraları üzerindeki biyokimyasal ve histopatolojik etkileri erkek Wistar sıçanlarında (n = 48) araştırılmıştır. Hayvanlar rastgele dört gruba ayrılmıştır: Sağlıklı Kontrol, Yanık Kontrol, Gümüş Sülfadiazin (1% krem) ve JAPL (bitkisel) krem. Tedaviler 21 gün boyunca topikal olarak günde bir kez uygulanmıştır. Biyokimyasal analizlerde, 21. günde JAPL grubunda süperoksit dismutaz (SOD) düzeylerinde anlamlı artış (89.3 ± 4.6 ng/mL; $p < 0.001$) ve malondialdehit (MDA) düzeylerinde belirgin azalma (142.5 ± 7.2 nmol/L; $p < 0.01$) saptanmıştır. Histopatolojik değerlendirmelerde ise 21. günde JAPL grubunda epitel rejenerasyonu, fibroblast proliferasyonu ve keratinizasyon belirgin şekilde gözlenmiştir. Bu bulgular, test edilen bitkisel formülasyonun ikinci derece yanıkların tedavisinde etkili olabileceğini göstermektedir; ancak, klinik etkinliğinin doğrulanması için ileri düzey insan çalışmalarına ihtiyaç vardır.

Anahtar kelimeler: Antioksidan savunma, *Argania spinosa*, fitoterapi, histopatoloji, ikinci derece yanıklar, *Juglans regia*.

INTRODUCTION

The skin, which covers a surface area of approximately 2 square meters in an adult and is the largest organ in the body, consists of two layers: the epidermis and the dermis. Underneath these layers are important skin accessory structures such as hair follicles, sweat glands and sebaceous glands, which are the sources of epithelial cells

(keratinocytes) that migrate to the clot and wound bed and play an important role in the wound healing process (Shpichka et al., 2019). In addition to maintaining the body's homeostasis, it also acts as a protective barrier against chemical, physical and bacterial threats (Miranda et al., 2024). Wounds resulting from trauma, cuts, tumors, poor blood flow, surgery, and especially burns, one of the most severe types of soft tissue damage, can disrupt the structure and functions of the skin. This disruption increases the risk of ulceration and infection (Basu et al., 2017; Oryan et al., 2017).

Burn injuries, affecting over 11 million people globally each year, pose significant public health concerns with both physical and psychological consequences (Hamilton et al., 2018; Makhmalzadeh et al., 2024). These injuries are caused by friction, extreme temperatures, radiation, chemicals, or electricity and lead to coagulative necrosis. Burns are generally classified by depth: first-degree (superficial, epidermal), second-degree (involving the epidermis and partial dermis), third-degree (involving complete destruction of the epidermis and dermis, often extending to the hypodermis) (Żwierełło et al., 2023; He et al., 2021), and deeper burns involving muscle or bone are classified as fourth-degree burns (Jenkins and Johnson, 2024). Deep second-degree burns are particularly problematic and often require surgical intervention due to extensive tissue loss and impaired healing capacity (Jeschke et al., 2020; Yadav et al., 2023). Inflammatory cascades and oxidative stress, primarily mediated by superoxide radicals and related reactive species, further exacerbate tissue damage (Sumsuzzman et al., 2020). These processes compromise cellular viability and increase fluid loss, which can lead to systemic complications such as dehydration, renal failure, and even organ dysfunction (Stanojcic et al., 2018).

Wound healing in burns follows a triphasic process: inflammation, proliferation, and remodeling (Mulholland et al., 2017; Thiruvoth et al., 2015). However, compared to other types of skin injuries, burns exhibit delayed progression due to persistent oxidative damage and microbial colonization (Stone et al., 2018; Wang et al., 2018). Hence, antioxidant interventions that neutralize free radicals have garnered attention as supportive therapies in burn care. Phytotherapeutic agents—derived from medicinal plants—are being explored for their wound healing properties, attributed to their bioactive compounds such as flavonoids, terpenes, saponins, and polyphenols (Mohanta et al., 2020; Bold et al., 2022). These agents have the potential to reduce oxidative stress and inflammation while promoting re-epithelialization and tissue remodeling.

In this context, a topical formulation containing *Juglans regia* leaf extract, *Argania spinosa* kernel oil, *Prunus dulcis* oil, and *Lavandula angustifolia* oil was developed and tested in a rat second-degree burn model. Each component was selected based on documented therapeutic properties: *J. regia* for its antimicrobial and antioxidant flavonoids (Ahuja et al., 2024), *A. spinosa* for its unsaturated fatty acids and terpenoids with skin-protective effects (Mechqoq et al., 2021), *P. dulcis* for its vitamin- and biotin-rich profile supporting dermal repair (Selwyn and Govindaraj, 2023), and *L. angustifolia* for its linalool-based anti-inflammatory and antibacterial potential (Prusinowska and Śmigielski, 2014; Wells et al., 2018).

Although these components have been individually studied, no prior work has assessed their synergistic efficacy in a standardized burn model using both biochemical and histological outcomes. Therefore, the present study was designed to test the hypothesis that this phytotherapeutic cream would enhance wound healing by modulating oxidative stress—specifically increasing SOD activity and reducing MDA levels—while simultaneously supporting epithelial regeneration and fibroblast proliferation.

MATERIAL AND METHOD

Materials used in the study: Ethanol (C_2H_5OH) was purchased from ISOLAB (Wertheim, Germany), ketamine was purchased from Richter Pharma (Wels, Austria), xylazine, formaldehyde, alcohol and xylene were purchased from Bayer (Istanbul, Türkiye), formalin-phosphate, hematoxylin-eosin were purchased from Sigma-Aldrich (St. Louis, Missouri, USA), MDA and SOD kits were purchased from SunRed Biotechnology (Shanghai, China), parafilm was purchased from Merck, *A. spinosa* kernel oil, *P. dulcis* oil and *L. angustifolia* oils were purchased from Bade Natural (Istanbul, Türkiye). *J. regia L.* leaf extract was obtained from Posof (Ardahan, Türkiye) and prepared as described. Cetearyl alcohol and Ceteareth-20 (7%) were purchased from Ataman Kimya A.Ş. (İstanbul, Türkiye) and used as emulsifiers in the cream formulation. All reagents were of analytical grade and used without further purification.

Preparation of cream formulation: For the preparation of the phytotherapeutic cream, *A. spinosa* kernel oil (15%), *P.dulcis* oil (15%), Cetearyl alcohol and Ceteareth-20 (7%), distilled water (55%), glycerin (4%), *J. regia* leaf extract (3%), and *L. angustifolia* essential oil (1%) were used.

The preparation followed standard cosmetic emulsification steps. The oil phase (oils and emulsifier) and aqueous phase (distilled water and glycerin) were separately heated to approximately 70°C using water baths. The oil phase was gradually added to the aqueous phase under continuous homogenization using a laboratory-grade magnetic stirrer (300 rpm) to form a uniform emulsion. Upon cooling to ~40°C, active ingredients (*J. regia* extract and *L. angustifolia* oil) were added and mixed for 15 minutes

to achieve a homogenous cream consistency. The final formulation was stored in sterile containers at 4°C and used fresh daily for application.

Procurement of Experimental Animals: "Local Ethics Committee approval has been obtained for animal experiments to be conducted and ethical principles have been followed."

The present study was conducted on (♂;male)Wistar-albino rats (n=48, 230±30 g) obtained from the Atatürk University Medical Experimental Application and Research Center (ATADEM) Ethics Committee in accordance with the decision numbered E-75296309-050.01.04-2400427040, dated 26.12.2024. During the 1-week period until the experimental phase, the rats were adapted to the environment in polycarbonate cages, under a 12:12-hour light/dark cycle, at a controlled temperature of 22±2°C, with a normal diet and ad libitum access to water (Palabıyık et al., 2023). The care and use of experimental animals was carried out in accordance with the decision numbered 193 in the national guidelines (Cinar et al., 2019).

Burn Model Preparation: Prior to experimental modeling, 48 male Wistar-albino rats (*Rattus norvegicus*, 230±30 g) were randomized into four groups (n = 12 per group):

1. Healthy Control (HC)
2. Burn Control (BC)
3. Silver Sulfadiazine (SS, 1% cream)
4. JAPL Cream (herbal formulation)

The second-degree contact burn model was performed under anesthesia induced by intraperitoneal (i.p.) administration of ketamine (40 mg/kg) and xylazine (5 mg/kg) (Palabiyik et al., 2024). Doses were calculated according to the body weight of each animal. The same protocol was used for burn induction, all intermediate procedures, and terminal sampling. After shaving and disinfection with povidone-iodine, a preheated brass comb (95°C) was applied to the dorsal surface without pressure for 15 seconds, creating homogeneous burns (~4 cm²). Care was taken not to affect the underlying muscle tissue. Wound areas were disinfected daily with iodine and sterile saline.

Treatment Protocol and Dosage: JAPL cream was applied topically at a dose of 0.5 g per wound area (approx. 4 cm²) once daily for 21 days using a sterile spatula. The same volume was applied each day to ensure dose standardization. No occlusive dressing was used. The SS group received 0.5 g of 1% silver sulfadiazine cream under identical conditions. All treatments began 24 hours post-burn induction. Untreated groups (HC, BC) received no topical intervention but underwent the same handling procedures. The application protocol was monitored by the same investigator to ensure consistency. The formulation was not tested for percutaneous absorption or penetration kinetics; thus, this remains a limitation of the current study.

Figure 1 illustrates the experimental workflow, including cream preparation, burn induction, and treatment application in the second-degree burn model.

Figure 1. Schematic Representation of Topical Cream Preparation, Burn Modeling, and Treatment Protocol.

Wound Healing: Macroscopic follow-up was performed during the experiment, wound images were photographed on days 0, 3, 7, 14 and 21 to evaluate wound healing rates and lesion sizes were determined. Percentage contraction levels in the burn skin tissue were determined with the researcher program to evaluate the changes in the wound areas. The following formula was used for this calculation (Bayir et al., 2019);

Percentage of wound contraction = (Initial wound size - Specific day wound size)/Initial wound size × 100

Taking Blood and Skin Samples: In order to perform biochemical and histopathological analyses, 3 randomly selected rats from each group were sacrificed on the 3rd, 7th, 14th and 21st days under appropriate anesthesia (ketamine (40 mg/kg) and xylazine (5 mg/kg), i.p.). Blood samples were collected from the thorax with a syringe and subjected to centrifugation (4000 rpm, +4°C, 10 min) (Palabiyik et al., 2024). The obtained sera were stored at -80°C for antioxidant enzyme activity measurement (superoxide dismutase (SOD)) and lipid peroxidation analysis (malondialdehyde (MDA)). The burn wound area was surgically removed together with the healthy tissue it was in contact with and washed and cleaned with cold saline. The samples were left in 10% formalin to be used in pathological tests (Palabıyık, 2022).

Lipid Peroxidation and Antioxidant Enzyme Activity: The activities of enzymes and free radical amounts in the antioxidant mechanism are determined by biochemical tests. In this study, SOD and MDA, which are oxidative stress parameters, were measured.

The analysis of SOD, which is the first line of defense against reactive oxygen species (ROS) produced in the cell and other such potentially dangerous molecules (Chidambaram et al., 2024), was performed with the Rat (SOD) ELISA Kit (Catalog No: 201-11-0169, SunRed); MDA, which plays an important role in the development of various processes including oxidative stress, endothelial dysfunction, cancer, inflammation and atherogenesis as one of the basic end products of lipid peroxidation (Ibrahim-Achi et al., 2023), was studied with the Rat (MDA) ELISA Kit (Catalog No: 201-11-057, SunRed) according to the given protocol. Results were expressed as ng/mL for SOD and nmol/L for MDA.

Histopathological Evaluation-Tissue Preparation for Light Microscopy: A sample containing both healthy and burned tissue was taken from the second-degree burn site, cassetted, and prepared for histopathological examination. Tissue processing was performed in four stages: formaldehyde, alcohol, xylene, and paraffin, over 13 hours. Tissues were embedded in appropriate molds, and 4 µm-thick sections were prepared using a microtome. These sections were first exposed to a water bath between 30°C and 45°C, then transferred to slides and incubated in an 80°C oven for 30 minutes. After immersion in xylene, the prepared tissue sections were stained with hematoxylin-eosin using a Leica ST5010 Autostainer XL. After staining, the slides were mounted using a Leica CV5030 Fully Automatic Glass Coverslip Mounting Machine. Histopathological analyses were performed using an Olympus BX60 light microscope equipped with 20x and 40x objectives (Sulumer et al., 2024).

Phytochemical Characterization: Methodological Constraints and Study Limitations: While the therapeutic potential of the selected plant-derived components has been extensively described in the scientific literature, no direct phytochemical or physicochemical characterization (e.g., HPLC, GC-MS, MS/MS) of the formulated cream was conducted in the present study to verify the presence, concentration, or stability of its active constituents. As such, mechanistic interpretations regarding the biological effects of specific compounds within the formulation rely primarily on established evidence from prior studies rather than empirical confirmation. This methodological limitation is explicitly acknowledged and discussed in the limitations section of this manuscript.

Statistical Data Analysis: Each experimental group at each time point included three biological replicates (n = 3 animals per group per time point), and no additional technical replicates were performed; each blood and tissue sample was analyzed once per biomarker. Data were analyzed separately for each time point (days 3, 7, 14, and 21) using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test to control the family-wise error rate. Statistical analyses were conducted using GraphPad Prism version 8.0.2 (GraphPad Software, San Diego, USA).

Significance thresholds were standardized and are reported as follows:

ns (not significant): $p > 0.05$

($p^* < 0.05$), ** ($p^* < 0.01$), *** ($p^* < 0.001$), **** ($p^* < 0.0001$)

A post-hoc power analysis performed on Day 21 MDA and SOD data ($\alpha = 0.05$) indicated Cohen's $f \approx 18.27$ and a statistical power of 1.00, confirming that the design was sufficient to detect large group differences despite the limited sample size. Detailed calculation methods are presented in Supplementary Methods, and a summary is provided in Supplementary Table 1. Supplementary Material.

Supplementary Methods: Power Analysis Calculation: A post-hoc power analysis was conducted using G*Power version 3.1 for a one-way ANOVA (fixed effects, omnibus test). The calculations were based on Day 21 SOD and MDA biomarker data (Cohen,2013):

MSwithin calculation:

$$SE_{\text{diff}} = \sqrt{\frac{2 \cdot MS_{\text{within}}}{n}} \Rightarrow MS_{\text{within}} = \frac{SE_{\text{diff}}^2 \cdot n}{2}$$

Eta-squared (η^2):

$$\eta^2 = \frac{SS_{\text{between}}}{SS_{\text{total}}}$$

Cohen's f:

$$f = \sqrt{\frac{\eta^2}{1-\eta^2}}$$

Power calculation:

ANOVA power was computed using Cohen's *f*, group count ($k = 4$), total sample size ($N = 12$), and $\alpha = 0.05$.

Results: Cohen's $f \approx 18.27$ and power ≈ 1.00.

Table 1. Post-hoc Power Analysis Results.

Biomarker	Day	Group Means (Mean ± SE*)	Pooled SD	Cohen's f	Power (1-β)
SOD	21	HC: 10.69 ± 0.60 BC: 3.09 ± 0.60 SS: 23.50 ± 0.60 JAPL: 31.40 ± 0.60	≈ 0.74	≈ 18.27	≈ 1.00
MDA	21	HC: 10.69 ± 0.60 BC: 3.09 ± 0.60 SS: 23.50 ± 0.60 JAPL: 31.40 ± 0.60	≈ 0.74	≈ 18.27	≈ 1.00

RESULTS

Lipid Peroxidation and Antioxidant Enzyme Activity: A progressive increase in SOD levels (ng/mL serum) was observed across all time points in the JAPL-treated group, with a particularly notable elevation on day 21 compared to day 3 ($p < 0.05$). This pattern suggests a robust and sustained antioxidant response induced by the JAPL formulation. Similarly, the SS group exhibited a statistically significant increase in SOD levels over time, though to a lesser extent than the JAPL group ($p < 0.05$ at day 21). In contrast, the BC group showed minimal variation across days, with consistently lower SOD values relative to both treatment groups ($p < 0.05$ for JAPL vs. BC and SS vs. BC on day 21). The HC group maintained relatively stable SOD levels across all time points, serving as a reference baseline. These findings indicate that both treatment modalities enhanced endogenous antioxidant defense mechanisms, with JAPL exhibiting superior efficacy in upregulating SOD activity over the 21-day treatment period (Figure 2).

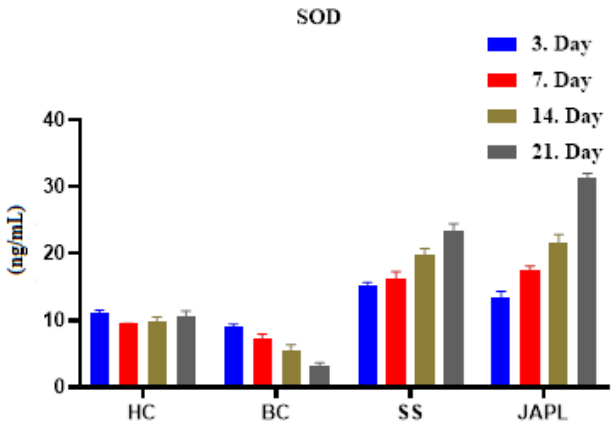

Figure 2. Changes in Superoxide Dismutase (SOD) levels (ng/mL serum) in the Dorsal Skin Burn Model in the Treatment Groups. * Statistical significance: ns = not significant ($p > 0.05$); * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

MDA levels (nmol/L serum)**,** a key biomarker of lipid peroxidation, were significantly elevated in the BC group at all time points, indicating persistent oxidative damage following thermal injury ($p < 0.001$ vs. HC for all days). In the SS group, although MDA levels remained high at the beginning of the treatment (day 3), a moderate but statistically significant reduction was observed by day 21 ($p < 0.05$ vs. day 3), suggesting partial amelioration of oxidative stress. In contrast, the JAPL-treated group demonstrated a marked and progressive decline in MDA levels from day 3 to day 21 ($p < 0.01$), with day 21 values significantly lower than both the BC and SS groups ($p < 0.001$ and $p < 0.05$, respectively). The HC group maintained consistently low MDA levels across all time points, as expected. Collectively, these results indicate that while both treatments attenuated lipid peroxidation, JAPL was more effective in mitigating oxidative damage, as evidenced by a greater reduction in MDA concentrations over the course of treatment (Figure 3).

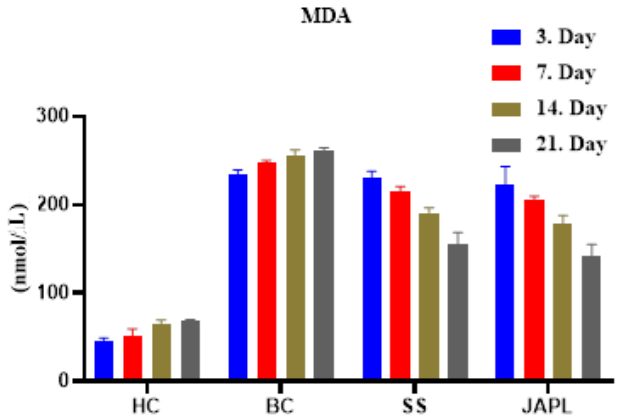

Figure 3. Changes in Malondialdehyde (MDA) levels (nmol/L serum) in Dorsal Skin Burn Model in Treatment Groups. * Statistical significance: ns = not significant ($p > 0.05$); * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Histopathological Evaluation-Tissue Preparation for Light Microscopy: Histological examination of skin tissues across experimental groups revealed marked differences in tissue damage, inflammation, and regeneration over the 21-day period. In the Healthy Control (HC) group, normal skin architecture was consistently preserved. Hematoxylin-eosin (H&E) stained sections displayed intact epidermis (green arrow), well-organized dermal appendages (white arrow), and underlying subcutaneous and muscular layers (yellow arrow), with no signs of inflammation or structural disruption (Figure 4).

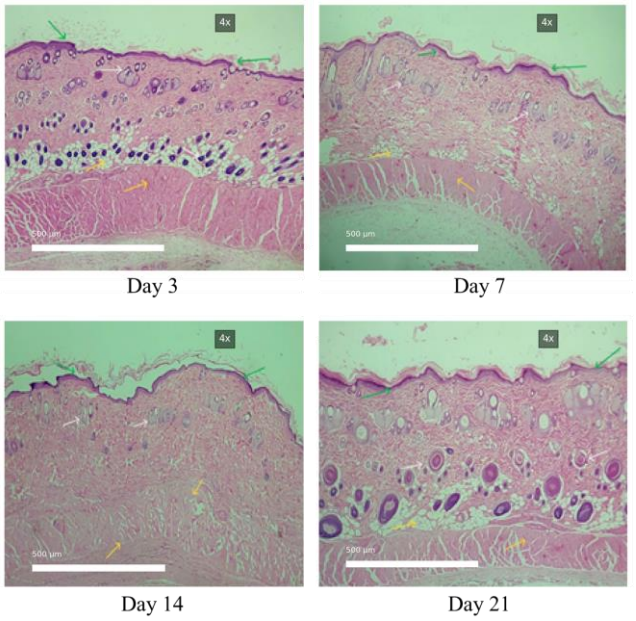

Figure 4. Histopathological Images of the Healthy Group, (H&E, x4).

In the Burn Control (BC) group, pronounced histopathological alterations were noted. On day 3, extensive epidermal loss, dermal eosinophilic degeneration (blue arrow), and dense inflammatory infiltration (red arrow) were evident. By day 14, intraepidermal vesicle formation (orange arrow) and absence of epithelial regeneration were prominent. Fibroblastic activation (light green arrow) emerged alongside localized calcification (yellow arrow), which persisted through day 21 despite partial epithelial regeneration. The persistence of inflammation and limited tissue remodeling suggest impaired healing (Figure 5).

The Silver Sulfadiazine (SS) group showed moderate improvement compared to BC. By day 7, partial regeneration of dermal structures, including hair follicles (white arrow), was observed. Epithelial re-epithelialization and keratinization (green arrow) began by day 14, though inflammation (red arrow) and fibroblastic proliferation (light green arrow) remained prominent. By day 21, epithelial integrity was largely restored, with reduced inflammation and minimal calcification (Figure 6). These findings indicate a delayed but progressing wound healing process under standard treatment.

In the JAPL-treated group, inflammation and fibroblastic activity dominated the early healing stages. On day 3, necrotic debris with underlying intense eosinophilic

degeneration and inflammatory infiltration were noted (red and blue arrows). Fibroblast proliferation (light green arrow) increased markedly from day 7 onwards. Although epithelial regeneration was not apparent on day 14, by day 21, a continuous regenerated and keratinized epithelium (green arrow) was observed, with active fibroblastic zones underneath. Notably, inflammatory cell infiltration was substantially diminished at this stage. These features suggest that JAPL treatment promoted a structured and progressive wound healing cascade, with active dermal remodeling and enhanced re-epithelialization (Figure 7). These findings are consistent with the histopathological scoring results shown in Table 2.

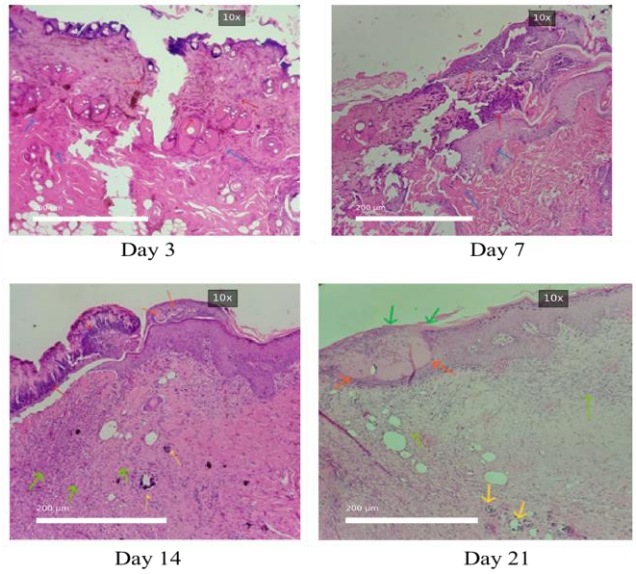

Figure 5. Histopathological Images of the Burn Group, (H&E, x10).

The semi-quantitative histopathological scoring results presented in Table 2 support the descriptive observations derived from H&E-stained sections and allow for comparative evaluation of healing dynamics among the groups (Alhilal et al., 2023). In the Burn Control (BC) group, prominent coagulation necrosis and severe inflammatory cell infiltration (scores: ++++ to +++++) persisted up to day 14, accompanied by high levels of edema and vesicle formation, but minimal fibroblastic activation or epithelial regeneration was observed. This profile suggests a prolonged inflammatory phase with limited tissue remodeling.

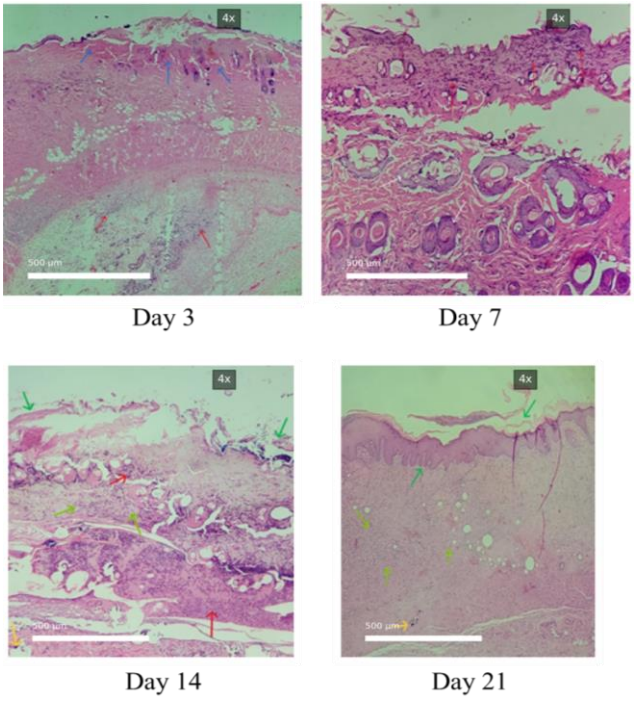

Figure 6. Histopathological Images of the Silver Sulfadiazine Group, (H&E, x4).

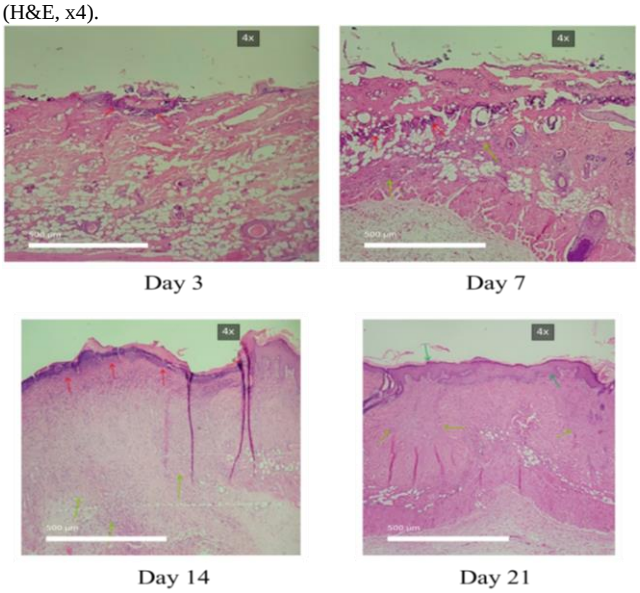

Figure 7. Histopathological Images of the Cream Treatment (JAPL) Group**,** (H&E, x4).

Table 2. Histopathological scores of burn and treatment groups

Histopathological parameters	**Burn Group**				**Silver Sulfadiazine Group**				**Cream Treatment (JAPL) Group**			
	Days				Days				Days			
	3.	7.	14.	21.	3.	7.	14.	21.	3.	7.	14.	21.
Eosinophilic degeneration	-	+	-	-	-	+	-	-	++	++	-	-
Coagulation necrosis	++++	++	-	-	++	++	-	-	++++	++	-	-
Inflammatory cells	++++	++++	+++++	+	++++	++	++++	-	+++	+++	+++	-
Edema	+++++	+	+	-	+++++	+	+	-	++	+	+	-
Vesicle/bullae	+	++	++	+	+	++	++	-	-	+	++	-
Fibroblastic activity	-	+	+	+	-	+	+++	+	-	+++	++++	++
Epithelial regeneration	-	-	-	++	-	-	+	++	-	-	-	++
Keratinization	-	-	-	+	-	-	+	+	-	-	-	+
-Negative, + very mild, ++ mild, +++ moderate, ++++ moderate/severe, +++++ severe												

In the Silver Sulfadiazine (SS) group, necrosis and edema decreased over time, with inflammation subsiding by day 21. Fibroblastic activation and epithelial regeneration scores gradually increased, indicating initiation of reparative processes. However, regeneration remained suboptimal compared to JAPL, with keratinization scores reaching only + or ++ by day 21. These findings are consistent with delayed but ongoing wound closure and partial restoration of skin integrity.

In contrast, the JAPL group demonstrated an accelerated and coordinated healing pattern. Coagulation necrosis and eosinophilic degeneration were initially high (++++), but sharply decreased by day 14. Importantly, fibroblastic activity reached its peak (++++) by day 14, with corresponding emergence of epithelial regeneration (++) and keratinization (+) by day 21. Inflammatory cell scores declined to baseline levels by the end of the study, highlighting effective resolution of inflammation. The early and intense fibroblast-mediated remodeling, combined with late-stage epithelial repair, underscores the superior regenerative effect of the JAPL formulation.

Overall, the histopathological scoring data quantitatively validate the visual tissue assessments and further emphasize that JAPL outperformed both untreated and standard treatment groups in promoting inflammation resolution, dermal matrix repair, and epidermal reconstitution within the 21-day treatment window.

DISCUSSION

Burn injuries are pathophysiological conditions characterized by a rapid increase in oxidative stress, which disrupts tissue integrity and initiates a cascade of local and systemic responses (Wang et al., 2023). This imbalance prolongs the inflammatory phase and delays wound healing. Balancing oxidative stress is therefore a critical factor for effective tissue repair and depends on the efficiency of endogenous antioxidant systems (Deng et al., 2021). In the present study, a cream formulation containing *J. regia* leaf extract, *A. spinosa* kernel oil, *P. dulcis* oil, and *L. angustifolia* oil (JAPL) was evaluated in a second-degree burn model. The results showed that JAPL modulated oxidative stress, promoted tissue regeneration, and outperformed silver sulfadiazine (SS) in both biochemical and histopathological outcomes.

From a biochemical perspective, superoxide dismutase (SOD) is a key indicator of antioxidant defense after burn injury (Zengin et al., 2025). In this study, SOD levels progressively increased in the JAPL group, reaching the highest values by day 21, indicating activation of endogenous defense mechanisms. Malondialdehyde (MDA), a marker of lipid peroxidation, was highly elevated in the burn control group but gradually decreased with JAPL treatment. Together, the increase in SOD and the decrease in MDA suggest that the formulation shifts the tissue environment toward antioxidant dominance, protecting cellular structures from oxidative damage. Previous studies have shown that phenolic compounds, flavonoids, and essential oils can provide similar effects by enhancing antioxidant capacity and stabilizing cell membranes (Hadi et al., 2024; Hu et al., 2025; Jamali and Kaboudanian Ardestani, 2024; Olivero-Verbel et al., 2024; Batiha et al., 2023; Selwyn and Govindaraj, 2023, Selvi, 2020; Altıntaş and Çelik, 2023; Özdenefe et al., 2023).

Histopathological evaluations reinforced the biochemical findings. The burn control group exhibited delayed epithelialization, persistent inflammation, and limited fibroblast activity. SS treatment provided partial improvement but showed less fibroblast proliferation and collagen organization. In contrast, JAPL-treated tissues demonstrated accelerated epithelialization, organized collagen fibers, and marked fibroblast activation by day 21. The combination of bioflavonoids and essential oils likely promoted fibroblast proliferation and extracellular matrix synthesis, contributing to faster and more stable wound closure (Addis et al., 2020; Melguizo-Rodríguez et al., 2021; Merecz-Sadowska et al., 2021).

Recent studies have also evaluated novel formulations combining antioxidant, anti-inflammatory, and healing-promoting agents (Comino-Sanz et al., 2021; Abdel-Mageed et al., 2025; Qureshi et al., 2022). For instance, a nano-emulsion comprising propolis, hyaluronic acid, and vitamin K demonstrated accelerated wound contraction, improved epithelization, fibrous tissue formation, collagen deposition, and enhanced angiogenesis in second-degree burns compared to control groups (Elsamman et al., 2024). Another recent work assessed a fibroblast-seeded hydrogel dressing in a deep partial-thickness burn model, showing that fibroblast proliferation and extracellular matrix regeneration are significantly enhanced in treated groups, similar to what we observed in our JAPL application (Novosad et al., 2024). Additionally, traditional herbal ointments have been compared to silver sulfadiazine in burn models, and outcomes suggest that formulations rich in flavonoids and essential oils can rival or surpass standard treatments in terms of antioxidant capacity and tissue remodeling (Skowrońska and Bazylko, 2023).

In conclusion, JAPL cream reduced oxidative stress, suppressed lipid peroxidation, and accelerated tissue regeneration in a second-degree burn model, achieving better outcomes than SS. By supporting both antioxidant protection and structural repair, the formulation shows potential as a plant-based therapeutic candidate for burn management. However, further experimental and clinical studies are required to confirm its efficacy and ensure safe translation to human applications.

Limitations: This study has several limitations that should be considered when interpreting the findings. First, the sample size was limited to three animals per group per time point, and no technical replicates were performed. Although our post-hoc power analysis (Cohen's f ≈ 18.27, power ≈ 1.00) confirmed adequate statistical power, smaller effect sizes may not have been captured. Second, phytochemical profiling of the cream formulation (e.g., HPLC, GC-MS) was not performed; mechanistic interpretations therefore rely on published bioactivity data of the ingredients rather than direct chemical analysis. These factors, along with the absence of a priori power calculation, limit the generalizability of the findings. Future studies with larger sample sizes, expanded time points, and comprehensive chemical analyses are recommended to validate these preliminary observations.

CONCLUSION

This study demonstrates that a phytochemical-enriched cream (JAPL), formulated with *J. regia* leaf extract, *A. spinosa* kernel oil, *P. dulcis* oil, and *L. angustifolia* oil, provides targeted therapeutic benefits in second-degree burn injuries by enhancing endogenous antioxidant defense and promoting structured tissue repair. JAPL significantly increased superoxide dismutase (SOD) activity while reducing malondialdehyde (MDA) levels, reflecting effective suppression of oxidative stress and lipid peroxidation at the cellular level. Histopathological assessments confirmed accelerated epithelialization, enhanced fibroblast activation, and organized collagen deposition, demonstrating active support of both the repair and remodeling phases of wound healing.

The findings of this study primarily contribute to wound healing research, burn injury management, and phytochemical-based therapeutic development by:

Providing preclinical evidence for a multi-component plant-derived cream that outperforms silver sulfadiazine in both biochemical and histopathological outcomes.

Offering mechanistic insights into how targeted antioxidant activation and tissue remodeling can synergistically improve burn wound healing.

Highlighting the translational potential of phytochemical formulations for developing safer, bioactive, and sustainable alternatives to conventional burn treatments.

While the absence of direct phytochemical characterization and the small sample size remain limiting factors, the mechanistic trends observed provide a strong foundation for future research. Next-stage studies should integrate compound-specific profiling, dose optimization, and expanded preclinical and clinical trials to validate efficacy, elucidate pharmacodynamic pathways, and support the translation of JAPL into clinical burn care.

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