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ORIGINAL ARTICLE

Harnessing the Anti-Inflammatory Potential of *Lamiaceae* Family Hydrolates: A Study of *Satureja Montana* and *Origanum Vulgare* in Burn Wound Management

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Abstract

Background/Aim: The management of severe thermal injuries is a significant clinical challenge, often leading to protracted inflammation, impaired tissue regeneration and increased susceptibility to infection, a situation exacerbated by growing antimicrobial resistance. Existing treatments frequently offer limited efficacy, creating a demand for novel treatment modalities. Aim of this study was to explore the therapeutic efficacy of innovative hydrogels formulated with hydrolates from two *Lamiaceae* family species, *Satureja montana* L and *Origanum vulgare* L, to modulate recovery after skin burn.

Methods: Standardised grade IIIa thermal burns were induced in twenty-five rabbits. The animals were then randomised into several cohorts for treatment: groups receiving topical applications of either *Satureja montana* L hydrolate gel, *Origanum vulgare* L hydrolate gel, or a standard therapeutic regimen. Control groups consisted of animals with untreated burns and healthy controls. To assess systemic inflammation, serum concentrations of key pro-inflammatory markers, interleukin-6, tumour necrosis factor-alpha and C-reactive protein, were quantified in serum via ELISA, while plasma levels of oxidative stress indicators (malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH)) were determined via specific colorimetric assay kits. Histopathological analysis (HE staining) assessed tissue regeneration and inflammation and IL-1β immunohistochemistry evaluated inflammatory.

Results: Fourteenth days post-injury, the cohort treated with *Satureja montana* L hydrolate exhibited a reduction in systemic inflammatory markers, approaching physiological baseline levels. Histological examination confirmed epithelialisation, markedly reduced inflammatory cell infiltration and robust inflammation reduction in this group. While *Origanum vulgare* L also showed beneficial effects, *Satureja montana* L consistently outperformed both its herbal counterpart and conventional treatments in promoting tissue repair and immunomodulation.

Conclusion: Presented findings indicate that phytotherapeutic preparations utilising hydrolates, especially the formulation derived from *Satureja montana* L, constitute a highly promising adjunctive strategy for the clinical management of thermal burn injuries.

Key words: Burns; *Lamiaceae*; *Satureja montana* L; *Origanum vulgare* L; Hydrolates; Phytotherapy; Tissue regeneration; Oxidative stress; Inflammation.

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Introduction

The skin, the largest organ of the human body, plays a critical role in maintaining homeostasis and protecting against external environmental factors. Its regenerative capacity is attributed to the cambial elements of the epidermal basal layer, skin appendages, as well as the activity of fibroblasts and macrophages, which enable effective remodelling and re-epithelialisation of damaged areas.[1]

Among numerous traumatic exposures, burn injury represents one of the most severe challenges to the regenerative potential of the skin. Burns, potentially resulting from thermal, chemical, electrical, or radiation causes, not only induce local damage but also trigger a cascade of systemic pathophysiological reactions, posing a serious burden on the healthcare system.[2, 3] Despite significant progress in burn treatment over the last decade, the search for effective therapies remains a pressing issue in modern medicine.

The pathomorphological picture of burn injury is characterised by the formation of a three-zone damage architecture: a central zone of coagulative necrosis, where irreversible denaturation of structural proteins of the epidermis and dermis occurs; a zone of stasis, surrounding the necrotic area, with progressive microcirculatory thrombosis; and a peripheral zone of hyperaemia, characterised by vasodilation and increased vascular wall permeability.[4] The cascading activation of the complement system, induction of a spectrum of pro-inflammatory mediators and different prostaglandins, initiates a powerful local inflammatory response with subsequent systemic dissemination of mediators.[5] These processes lead to compromise of the epidermal barrier, dysregulation of transepidermal water loss and the creation of a favourable microenvironment for bacterial adhesion and biofilm formation, which collectively contribute to a protracted healing process, delayed epithelialisation, formation of incompetent granulation tissue and a significant risk of secondary infectious complications capable of leading to multiple organ dysfunction.[6, 7] Existing treatment methods, including the use of antiseptic, antibacterial and anti-inflammatory agents, have limited effectiveness as they primarily act on isolated nodes of pathogenesis and do not provide comprehensive control of inflammation and stimulation of regeneration.[8]

Within the complex microbial ecology of burn wounds, *Staphylococcus aureus* rapidly colonises the moist, protein-rich burn eschar, forming highly resilient biofilms. Its arsenal of toxins, quorum-sensing-regulated virulence factors and intrinsic and acquired resistance to multiple antibiotic classes make it a primary driver of delayed wound closure, graft failure, sepsis and increased mortality in burn patients.[9] Current standard guidelines emphasise early excision, timely wound coverage and infection control; however, conventional topical regimens often fail to sufficiently disrupt *S aureus* biofilm-mediated persistence or adequately support coordinated tissue regeneration.[10] This highlights a critical necessity for developing of innovative therapeutic approaches capable of tackling these complex challenges posed by burn wound infections, particularly those involving resistant pathogens.

One possible solution to this problem may be phytotherapeutic medicinal products (PMPs), which account for about 40 % of all available medicines.[11] Of particular interest in this context are the medicinal plants from *Lamiacea* family, known for synergistic spectrum of pharmacological metabolites: polyphenolic acids (rosmarinic acid, caffeic acid), flavonoid glycosides (quercetin, luteolin) and terpenoid components of essential oils (carvacrol, thymol).[12] These compounds determine their pleiotropic pharmacodynamic effects on inflammatory-reparative processes. Specifically, carvacrol, gamma-terpinene and p-cymene exhibit pronounced antibacterial, immunomodulatory, pro-regenerative and antioxidant properties.

For the phytotherapeutic treatment of burn wounds, one of the most rational dosage forms is hydrolates – aqueous extracts obtained by steam distillation.[13] This method allows for the preservation of a high concentration of essential oils while being cost-effective. Unlike essential oils, hydrolates primarily consist of oxygenated monoterpenes and contain water-soluble phenolic compounds and low-molecular-weight peptides, which accounts for their lower membrane toxicity, differences in antimicrobial action mechanisms, improved skin tolerability and potential for synergistic effects, making them promising for topical application in burn injuries.[14]

Main hypothesis of this study was that phyto-preparations formulated as hydrolates from *Satureja montana* L and *Origanum vulgare* L possess the ability to accelerate the regenerative processes in thermally damaged skin, partly due to the modulation of local inflammatory status, pro-regenerative and antioxidant properties. Aim of study was to analyse the effect of topical treatment with *Satureja* and *Origanum* hydrolates based on key healing processes in third-degree burns, specifically their ability to modulate local inflammation and accelerate re-epithelialisation.

Methods

Experimental animals

Twenty-five male New Zealand White rabbits (3.0 kg ± 0.33 kg) and aged 7 – 8 weeks were utilised at the study's commencement. To ensure proper acclimatisation and health monitoring, all animals underwent a 14-day quarantine period prior to experimental procedures.

All rabbits maintained under controlled environmental conditions: ambient temperature between 22 – 23 °C, relative humidity ranging from 40 – 60 % and a precisely regulated life cycle. The food consisted of a standard laboratory rabbit diet, including unlimited water (*ad libitum*).

Experimental design

To minimise bias and enhance objectivity, animals were randomly assigned to one of five experimental groups, with five rabbits allocated to each group using a simple randomisation method (Figure 1):

- Control group (I), five rabbits served as a baseline control;
- Group II (untreated burn): five rabbits with burn wound without treatment intervention;
- Group III (standard care): five rabbits received thermal wound followed by treatment, which included applications of "Levomekol" ointment (containing methyluracil and chloramphenicol), boric acid and gauze dressings impregnated with "Betadine" (povidone-iodine);
- Group IV (*Satureja montana* L): five rabbits with thermal wound managed with a hydrolate derived from *S montana* L;
- Group V (*Origanum vulgare* L): five rabbits with thermal wound managed with a hydrolate derived from *O vulgare* L.

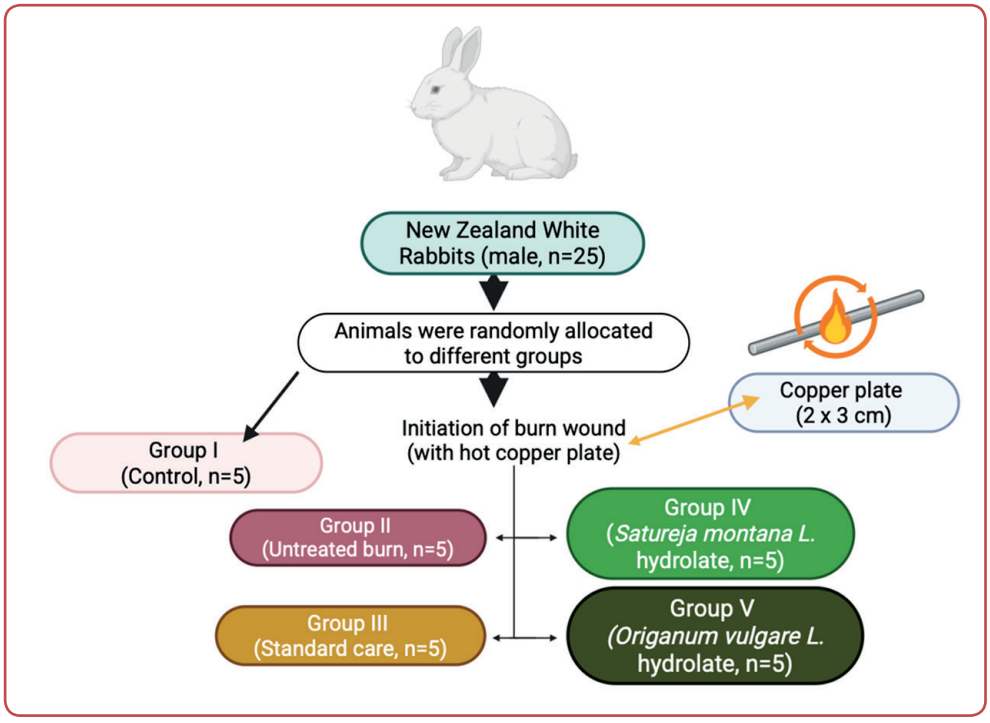


Figure 1: Overview of experimental groups and treatment allocation

The key points of the study were baseline (starting point), 3 days, 7 days and 14 days. Initial evaluations encompassed comprehensive assessments of body weight, behavioural responses and biochemical parameters. In instances where animals experienced unresolvable complications or reached predefined humane endpoints, euthanasia was performed using a combination of Zoletil® 100 (15 mg/kg) and xylazine (5 mg/kg).

Burn injury induction

Prior to the procedure, dorsal fur was removed. Animals were anesthetised with an intramuscular injection of Zoletil 100 (15 mg/kg). A standardised third-degree "a" (IIIa) thermal burn was induced by hot copper plate (6-inch bar) to the depilated dorsal surface for 30 seconds.

Preparation and characterisation of hydrolates

Authenticity of the medicinal plants *Satureja montana* L and *Origanum vulgare* L was verified by a qualified taxonomist and representative voucher samples were archived at the Herbarium of the Institute of Translational Medicine and Biotechnology, Sechenov University (Moscow, Russia).

For the production of hydrolates, freshly collected aerial parts of both species were processed using steam distillation under controlled laboratory conditions. Each portion of plant material (approximately 1.5 kg) was distilled in deionised water at a 1:5 plant-to-solvent ratio, employing a Clevenger-type apparatus equipped with a temperature-regulated heating unit and condensation system. Distillation was carried out for roughly three hours at atmospheric pressure, resulting in a condensate composed of aqueous and oil fractions. The hydrophilic distillate (hydrolate) was carefully separated from the essential oil layer and retained for subsequent analysis and formulation.

Chemical composition of hydrolates

The volatile constituents of the *Satureja montana* L and *Origanum vulgare* L hydrolates were analysed by gas chromatography coupled with mass spectrometry (GC–MS). The chromatographic profiles demonstrated a rich and diverse spectrum of volatile metabolites, primarily composed of oxygenated monoterpenes, which are emblematic for species of *the Lamiaceae* family.

Although both hydrolates exhibited comparable qualitative composition, quantitative differences were identified in the relative proportions of their principal components: carvacrol, thymol and p-cymene. Minor shifts in retention times and relative intensities were also recorded, which can be attributed to natural variability in the botanical material and slight differences in distillation conditions between batches.

A concise summary of the identified constituents and their relative abundance is provided in Tables 1 and 2.

Table 1: Chemical composition of Satureja montana L hydrolate

Compound	Retention index (RI)	Concentration (%)
Carvacrol	1304	86.7
Thymol	1281	3.2
p-Cymene	1018	3.5
Linalool	1092	1.3
γ-Terpinene	1052	1.4
Camphor	1143	tr

Notes: RI values were calculated relative to n-alkanes; concentrations are expressed as relative peak area percentages; tr – indicates trace amounts (< 0.1 %)

Table 2: Chemical composition of Origanum vulgare L hydrolate

Compound	Retention index (RI)	Concentration (%)
Carvacrol	1296	41.5
Thymol	1290	19.3
p-Cymene	1029	8.6
Linalool	1060	8.1
γ-Terpinene	982	0.5
Camphor	951	tr

Notes: RI values were calculated relative to n-alkanes; concentrations are expressed as relative peak area percentages; tr – indicates trace amounts (< 0.1 %)

Product safety and standardisation

Distillation parameters (time, temperature, pressure) were strictly controlled. Each hydrosol batch was validated for quality, including transparency (visual), pH: 3.0 – 6.0 (*Hanna Instruments*, USA), density (*Anton Paar GmbH*, Austria) and refractive index (*ATAGO Co Ltd.,* Japan).

Therapeutic gel formulation

A 2 % (20 g/L) hydroxyethyl cellulose (HEC) (*Sigma-Aldrich*, USA) gel base was prepared. HEC was dispersed into the hydrosol with continuous mag-

netic stirring at room temperature, followed by gentle heating to 39 °C in a water bath to ensure complete hydration. The final gels were stored at 4 °C.

Biochemical assay

For analysis, blood was processed to obtain two fractions. Serum was collected in plain tubes after clotting and centrifugation, while plasma was obtained from EDTA-anticoagulated tubes centrifuged under identical conditions. All aliquots were stored at −80 °C until use. Concentrations of interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α) and C-reactive protein (CRP) were quantified in serum. IL-6 and TNF-α were measured using species-specific sandwich ELISA kits, while CRP was determined with a commercial ELISA kit. All assays were performed in duplicate according to the manufacturers' protocols.

To assess oxidative stress, plasma levels of malondialdehyde (MDA), superoxide dismutase (SOD) and reduced glutathione (GSH) were determined using specific colorimetric assay kits. All assays were performed in duplicate according to the manufacturers' protocols, with optical density measured using a microplate reader.

Morphological assay

Tissue samples were fixed in 10 % neutral buffered formalin, processed and embedded in paraffin. Sections (3 µm) were stained with haematoxylin and eosin (HE) for general morphology.

Immunohistochemical (IHC) analysis

Immunohistochemical staining for interleukin-1β (IL-1β) was conducted on paraffin-embedded tissue sections using primary polyclonal antibodies against IL-1β (*ThermoFisher*, 1:100, USA). Visualisation was achieved with the HiDef Detection™ HRP Polymer system (*Cell Marque*, USA) and DAB substrate. The slides were counterstained with Mayer's hematoxylin. Quantification of IL-1β expression involved semi-quantitative scoring of staining intensity and distribution. All procedures were performed on an automated stainer (*Ventana Benchmark XT*) utilising the ultraView Universal DAB Detection Kit.

Statistical analysis

All quantitative data were processed and analysed utilising the STATISTICA software suite (V.13.5). The results are expressed throughout the manuscript as the mean ± standard deviation (SD). An initial assessment of data distribution was performed for all variables using the Shapiro-Wilk test to determine normality. Based on this assessment, non-parametric statistical methods were selected for intergroup comparisons. Differences among multiple experimental groups were evaluated with the Kruskal-Wallis test, followed by Dunn's post-hoc test for multiple comparisons adjustment. For direct comparisons between two specific groups, the Mann-Whitney U-test was employed. A p-value of less than or equal to 0.05 was considered the threshold for statistical significance in all analyses.

Results

Biochemical assay

The control group maintained stable baseline levels throughout the study, serving as a reference for physiological norms. On day 3 post-injury, a systemic inflammatory response was evident across all infected burn groups (II – V), most pronounced in the untreated group (Figure 2, 3). Inflammatory cytokines (IL-6, TNF-α) and acute-phase protein (CRP) in group II peaked significantly ($p < 0.001$ vs baseline). This acute phase was also characterised by oxidative stress, with MDA levels in group II reaching 11.9 ± 1.7 nmol/mL, while antioxidant defences (SOD: 83 ± 4.2 U/mL, GSH: 2.1 ± 0.3 µmol/L) were markedly suppressed ($p < 0.001$ vs baseline).

In contrast, both hydrolate-treated groups demonstrated an attenuated inflammatory and oxidative response from day 3 onwards. Group IV consistently exhibited the most pronounced reductions in inflammatory markers (eg IL-6: 23.4 ± 6.1 pg/mL; CRP: 26.1 ± 3.0 mg/L) and oxidative stress (MDA: 6.7 ± 1.4 nmol/mL), alongside better-preserved antioxidant capacity (SOD: 152 ± 8.5 U/mL; GSH: 4.2 ± 0.2 µmol/L) compared to group II ($p < 0.01$). Group V showed similar, though slightly less pronounced, improvements. The standard care group demonstrated moderate reductions in these markers compared to group II but generally performed less effectively than the hydrolate groups.

By day 7, group IV maintained lower levels of inflammatory cytokines and acute-phase proteins (eg IL-6: 25.7 ± 4.1 pg/mL; CRP: 18.0 ± 5.2 mg/L) and continued normalisation of oxidative stress

markers (MDA: 7.2 ± 0.8 nmol/mL; SOD: 167 ± 18.0 U/mL; GSH: 3.8 ± 0.3 µmol/L), outperforming group II ($p < 0.01$; Figure 2, 3). Group V also showed sustained improvement, with markers lower than group II ($p < 0.05$).

By day 14, group IV showed levels of inflammation and oxidative stress markers that were close to control values, indicating almost complete elimination of systemic inflammation and oxidative damage (Figure 2, 3). Group V also showed improvement. In contrast, group II showed a persistent increase in the levels of markers of inflammation and oxidative stress, indicating ongoing inflammation and oxidative damage (although after two weeks there was a persistent decrease in all indicators).

Morphological assay

Macroscopic findings. Across all experimental cohorts, the excised thermal burn zones uniformly presented as a dense, dry, reddish-brown eschar. By seven days, a small amount of greenish-yellow exudate was observed on the burn wound surface in groups II and III, persisting partially until the experiment's conclusion. Conversely, in animals from *Lamiacea* hydrogels groups, especially for fourth group, macroscopic signs of thermal healing, including complete defect replacement, were evident by day 7 and fully achieved by day 14, with no signs of infection. The worst healing scenario was observed in the group of rabbits without therapy.

Microscopic findings. On day 3, histological analysis of the burn site across all animal cohorts ini-

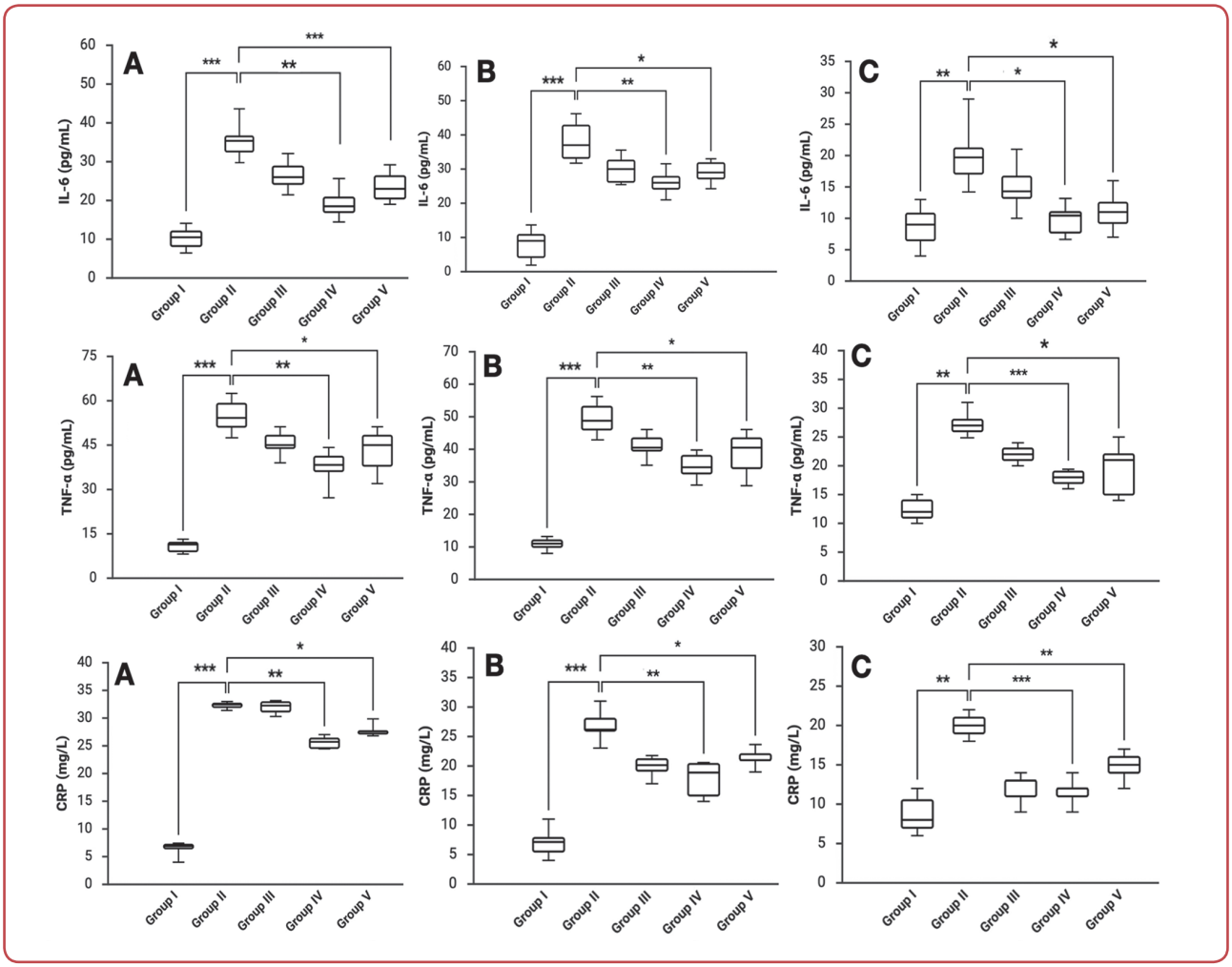


*Figure 2: The temporal variations in blood levels of interleukin 6 (IL-6), tumour necrosis factor-α (TNF-α) and C-reactive protein (CRP). Control group (I): baseline control; Group II (untreated burn): rabbits with burn wound without treatment intervention; Group III (standard care): rabbits received thermal wound followed by treatment, which included applications of "Levomekol" ointment, boric acid and gauze dressings impregnated with "Betadine"; Group IV (Satureja montana L): rabbits with thermal wound managed with a hydrolate derived from S montana L; Group V (Origanum vulgare L): rabbits with thermal wound managed with a hydrolate derived from O vulgare L;. Temporal dynamics were assessed at 3 (A), 7 (B) and 14 (C) days after the thermal injury. All data are expressed as mean ± SD. Statistical significance between groups is denoted by asterisks, where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$;*

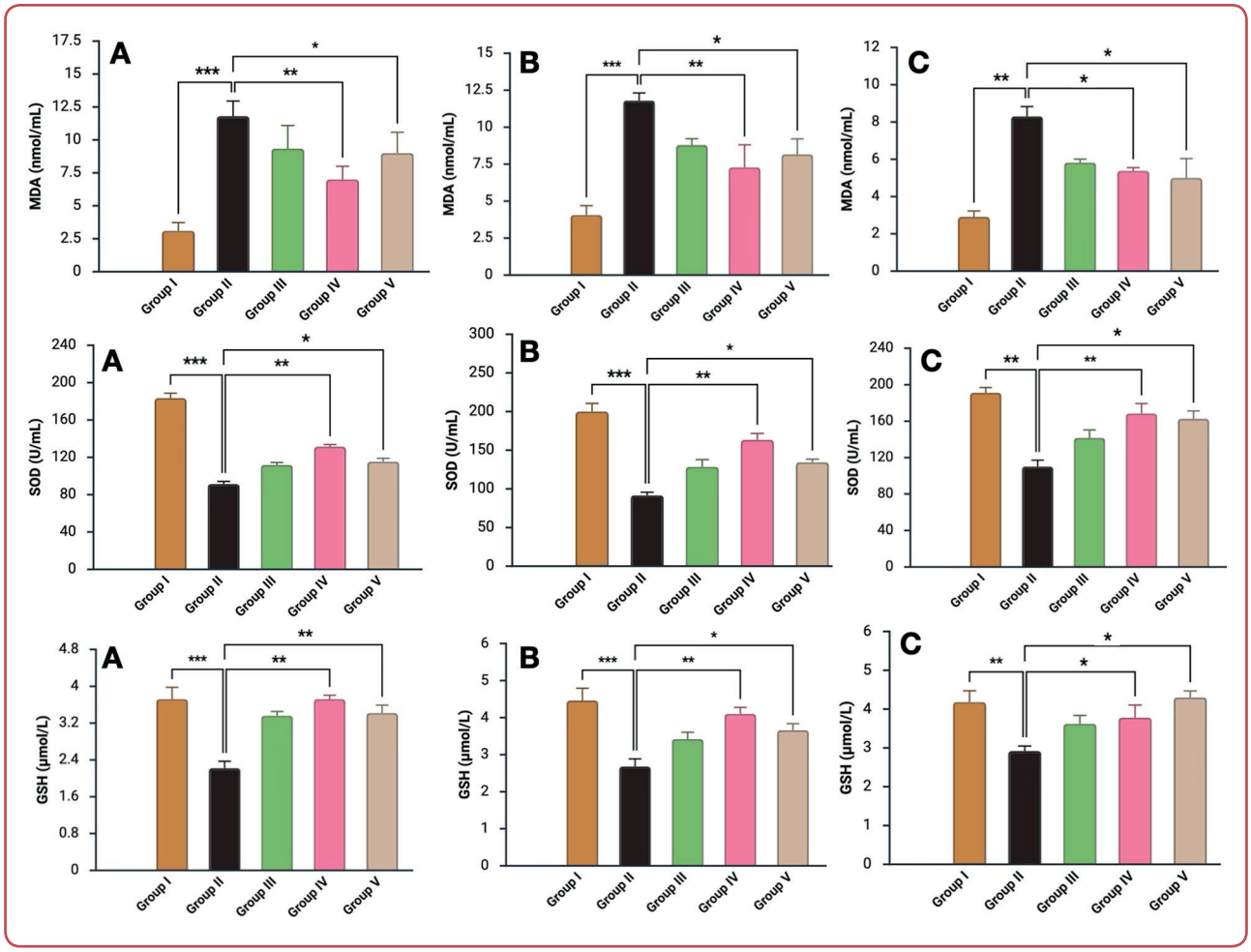
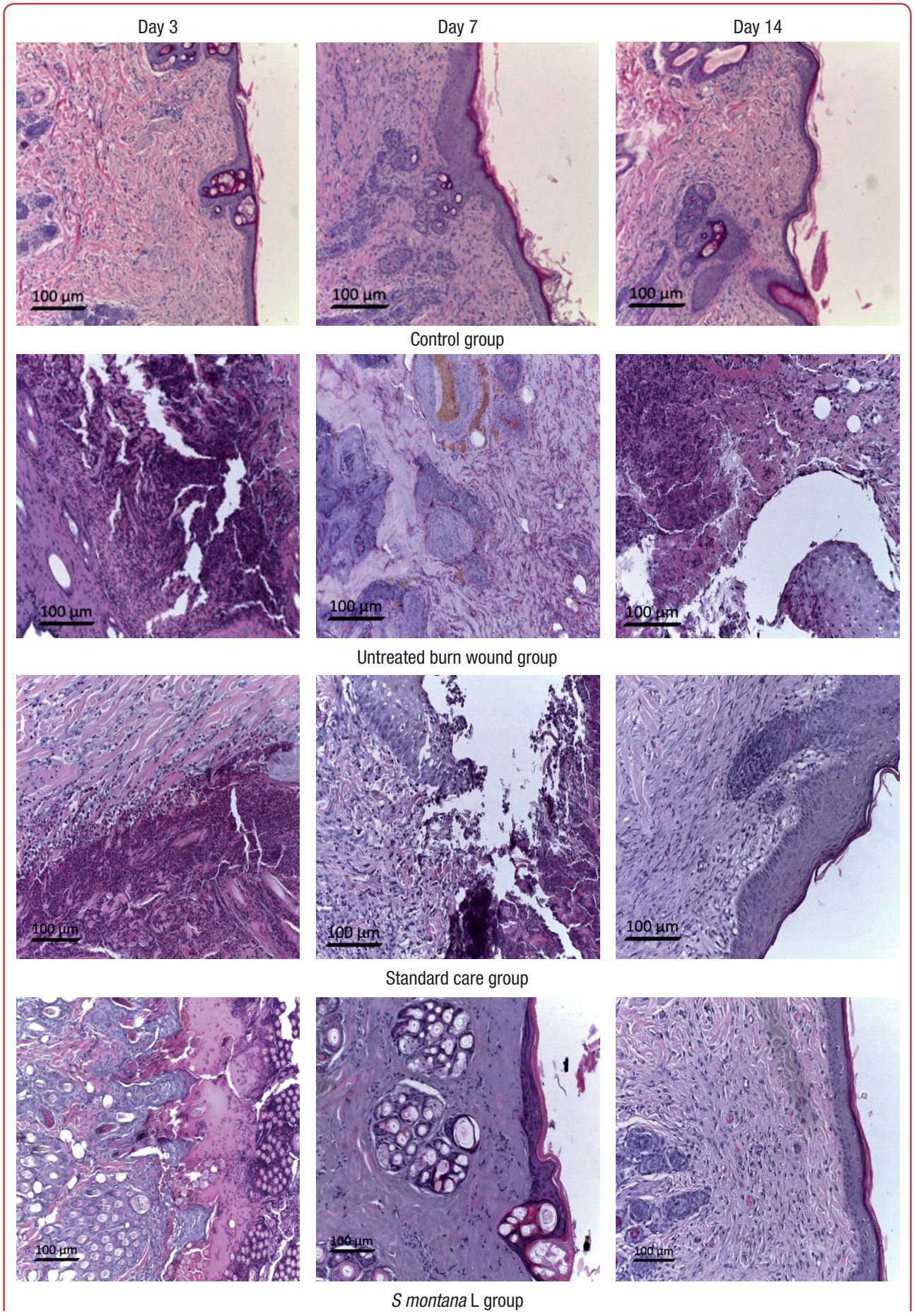


*Figure 3: The temporal variations in blood levels of malondialdehyde (MDA), superoxide dismutase (SOD) and reduced glutathione (GSH). Control group (I): baseline control; Group II (untreated burn): rabbits with burn wound without treatment intervention; Group III (standard care): rabbits received thermal wound followed by treatment, which included applications of "Levomekol" ointment, boric acid and gauze dressings impregnated with "Betadine"; Group IV (Satureja montana L): rabbits with thermal wound managed with a hydrolate derived from S montana L; Group V (Origanum vulgare L): rabbits with thermal wound managed with a hydrolate derived from O vulgare L; Temporal dynamics were assessed at 3 (A), 7 (B) and 14 (C) days after the thermal injury. All data are expressed as mean ± SD. Statistical significance between groups is denoted by asterisks, where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$;*

tially showed necrotic debris and a widespread inflammatory infiltrate, which was primarily composed of granulocytes and monocytes. Additionally, early signs of healing were observed, including nascent pockets of granulation tissue and significant vascular hyperaemia. The nuclei of epithelial cells at the wound margin also displayed reactive morphological changes.

On the seventh day post-injury, all experimental groups (II – V) displayed clear histological signs of active wound repair. Notably, the cohort receiving the *S montana* L hydrolate treatment demonstrated the most advanced regenerative activity, characterised by intense fibroblast proliferation and focal marginal re-epithelialisation. The therapeutic effects in groups III (standard care) and V (*O vulgare* L) were less remarkable, showing more moderate progress. In stark contrast, the untreated control animals exhibited the slowest healing trajectory, with minimal evidence of effective tissue regeneration or remodelling at this time point.

By day 14, burn wounds in group IV rabbits were almost completely regenerated: near-complete tissue restoration, evidenced by extensive re-epithelialisation and a dramatic reduction in dermal inflammatory infiltrates. Group V also showed healing effect, though some focal lymphoid infiltration remained in the subepidermal region. In comparison, the standard care group displayed only moderate effect, which was accompanied by persistent inflammation, while the untreated group showed only the very beginning of epithelial recovery (Figure 4).


Day 3
Day 7
Day 14
100 µm
Control group
Untreated burn wound group
Standard care group
S montana L group

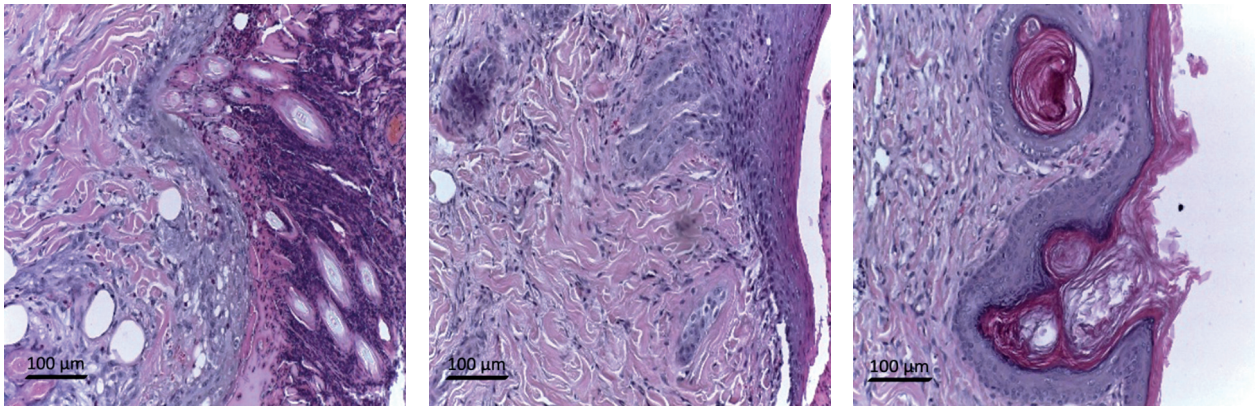


Figure 4: Microscope slides of burn wounds in experimental animals in all groups on all experimental time-points. Stained with haematoxylin and eosin (HE), magnification x200

Immunohistochemical analysis

Immunohistochemical analysis of IL-1β expression revealed distinct patterns across experimental groups and time points (Figure 5, 6). On day 3 post-injury, both the untreated burn wounds (Group II) and *S montana* L treated wounds showed a moderate presence of IL-1β positive cells, primarily localised to inflammatory infiltrates at the wound periphery. This indicated an early inflammatory response in both conditions. By day 7, a notable divergence in IL-1β expression was observed. In untreated burn wounds,

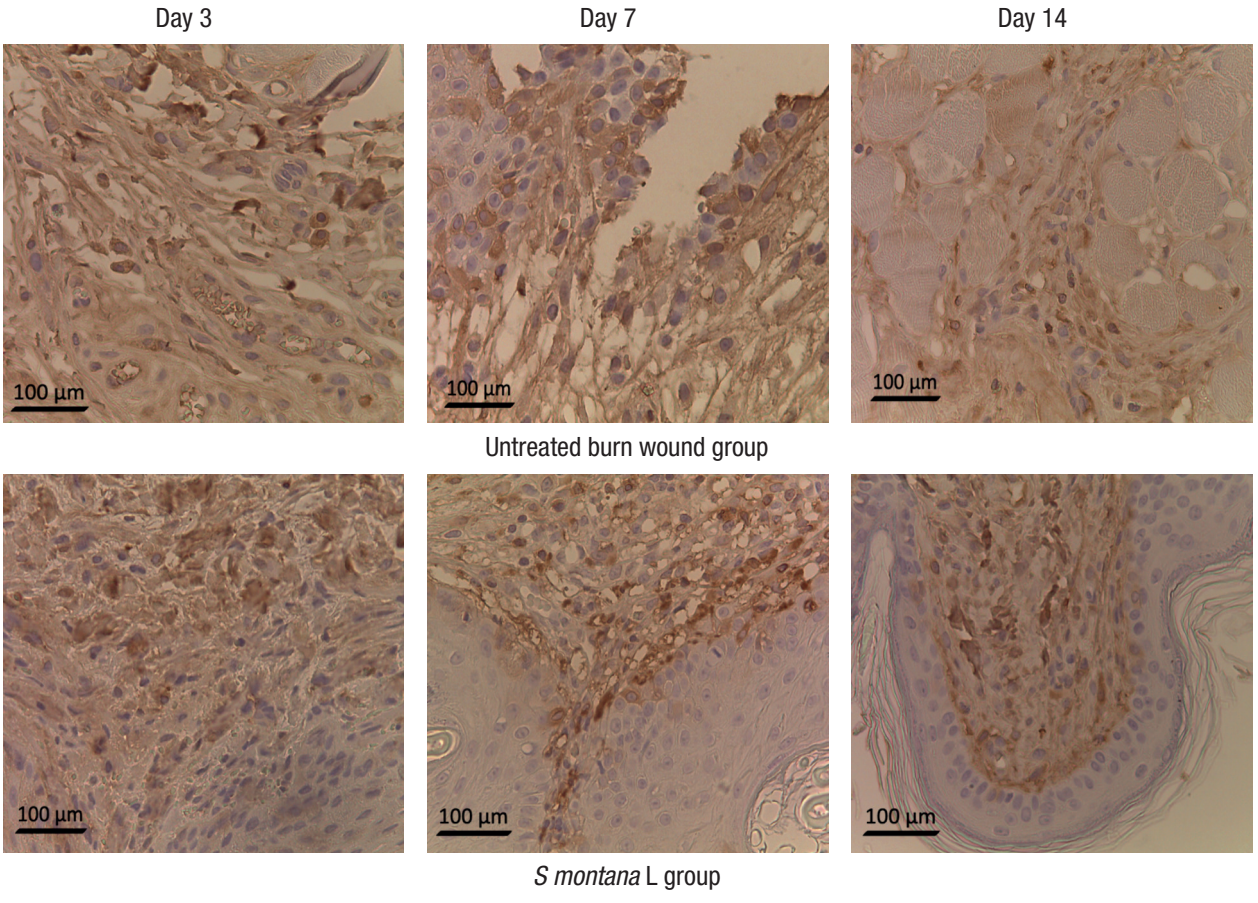


Figure 5: Immunohistochemical staining for IL-1β in burn wounds of untreated animals (Group II) and Satureja montana L treated animals (Group IV) on all experiment time-points, highlighting key differences inflammatory response, magnification x200

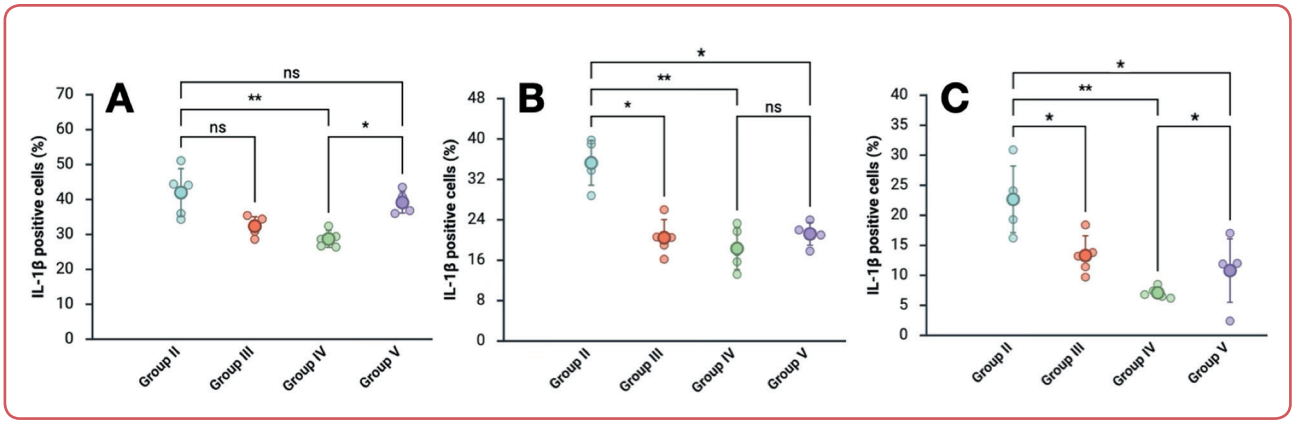


*Figure 6: Quantitative analysis of IL-1β positive cells in burn wounds across all experimental groups. Group II (untreated burn): rabbits with burn wound without treatment intervention; Group III (standard care): rabbits received thermal wound followed by treatment, which included applications of "Levomekol" ointment, boric acid and gauze dressings impregnated with "Betadine"; Group IV (Satureja montana L): rabbits with thermal wound managed with a hydrolate derived from S montana L; Group V (Origanum vulgare L): rabbits with thermal wound managed with a hydrolate derived from O vulgare L; Temporal dynamics were assessed at 3 (A), 7 (B) and 14 (C) days after the thermal injury. All data are expressed as mean ± SD. Statistical significance between groups is denoted by asterisks, where ns – not significant, * $p < 0.05$, ** $p < 0.01$;*

IL-1β positive cells remained scattered and often associated with necrotic areas, suggesting a persistent, dysregulated inflammatory state. In contrast, *S montana* L treated wounds exhibited a more organised pattern of IL-1β positive cells, concentrated in areas of active granulation tissue formation and re-epithelialisation, indicating a modulated inflammatory response conducive to healing. Group III showed a similar, but slightly less pronounced, reduction in IL-1β positive cells compared to Group IV, while Group V (*O vulgare* L) demonstrated IL-1β expression levels comparable to Group III, suggesting moderate anti-inflammatory effects.

By day 14, untreated burn wounds still presented with significant, albeit reduced, IL-1β positive cells, often associated with chronic inflammation and delayed healing. Conversely, *S montana* L treated wounds showed a decrease in IL-1β positive cells, with expression largely confined to residual inflammatory foci, signifying effective resolution of inflammation and advanced tissue regeneration. Group III and Group V maintained intermediate levels of IL-1β expression, superior to Group II but not reaching the optimal reduction seen in Group IV.

Discussion

The present investigation was designed to elucidate the wound-healing properties of hydrogel formulations containing hydrolates from two *Lamiaceae* species, *Satureja montana* L and *Origanum vulgare* L. Study's primary focus was to assess their capacity to modulate the complex processes of inflammation and tissue repair within a two-week timeframe using a rabbit model of third-degree thermal injury.

Thermal skin injuries represent a global health challenge, characterised by high morbidity and potential mortality. Epidemiological studies report approximately 8 million new burn cases annually worldwide, resulting in around 111,000 fatalities.[15] The burden is dramatically high in low- and middle-income countries, where limited access to specialised care contributes to higher incidence, complication rates and mortality.[8] Despite advancements in burn treatment protocols (especially second- and third-line pharmacological therapy), the issues of disability and pathological scar formation remain pressing, necessitating novel approaches in regenerative medicine.

The pathology of a burn wound is a dynamic and multiphasic process. The core of the wound comprises a zone of coagulation, where cellular death is irreversible. This is encircled by a zone of stasis, a critical area of ischemic tissue where cellular viability is compromised and the risk of progressive necrosis is high. The outermost region is the zone of hyperaemia, which is characterised by vasodilation. Crucially, the progression of this local injury is driven by two interconnected pathogenic events: the profound activation of

both local and systemic inflammatory pathways and the induction of severe oxidative stress.[4]

This inflammatory cascade is characterised by a rapid surge in signalling molecules. This includes a massive liberation of key pro-inflammatory cytokines and various chemokines. These molecules act as powerful chemoattractants, orchestrating the large-scale recruitment of leukocytes and other immune cells directly to the site of injury.[3] While an acute inflammatory response is integral to healing, its excessive or prolonged activation can exacerbate tissue damage, delay re-epithelialisation and contribute to hypertrophic scar formation.[16] Concurrently, burn injury induces an imbalance between reactive oxygen species (ROS) production and antioxidant defence, leading to oxidative stress. Excess ROS damages cell membranes, proteins and nucleic acids, worsening tissue damage and perpetuating chronic inflammation.[17] Presented results align well with these established pathophysiological mechanisms. Early in the experiment (day 3), pronounced signs of acute inflammation were observed: macroscopically, the burn zone in all groups was dry, dense and reddish-brown, while serum levels of IL-6, TNF-α and CRP were elevated. These changes reflect the typical systemic inflammatory response phase described in the literature.

In the search for novel therapeutic agents for burn wounds, *Satureja* and *Origanum* genera (*Lamiaceae* family) have attracted considerable attention. *S montana* (winter savoury) and *O vulgare* (oregano) are traditionally used in folk medicine and are actively investigated in modern pharmacology due to their rich phytochemical composition and broad biological activity.[18, 19] Their chemical profiles are characterised by a high content of phenolic monoterpenes, predominantly carvacrol and thymol, known for potent antioxidant, anti-inflammatory and antimicrobial properties.[20] Other bioactive components like γ-terpinene, p-cymene, linalool and 1,8-cineole may act synergistically to enhance overall therapeutic effects.[21]

A crucial aspect determining therapeutic potential is the extract form. Presented study focused on hydrolates (aqueous phases obtained during steam distillation for essential oil production). Unlike essential oils, which are concentrated lipophilic mixtures of volatile compounds that can cause irritation or sensitisation on damaged skin, hydrolates contain water-soluble components and significantly lower concentrations of volatile substances.[22] This makes them milder and safer for skin application, especially on high-sensitive and damaged skin after thermal trauma. Specialised literature indicates that hydrolates, despite lower concentrations of main components compared to essential oils, retain significant biological activity, including antioxidant and anti-inflammatory properties, due to hydrophilic compounds not transferring into the essential oil.[23]

Treatment with *S montana* L hydrolate gel attenuated the biochemical inflammatory markers, bringing them close to baseline by day 14. *O vulgare* L also reduced inflammation, albeit to a lesser extent. This anti-inflammatory effect aligns with the known properties of rosmarinic acid and thymol, major bioactive compounds in these plants, which have demonstrated capacity to modulate inflammatory pathways.[24] The sustained elevation of CRP in the untreated group underscores the detrimental impact of prolonged inflammation on wound resolution, a well-established factor in delayed healing and increased infection risk.

Histological and immunohistochemical evaluations constituted a holistic view of tissue repair. Macroscopically, *Lamiacea* treated groups showed earlier signs of healing and complete defect replacement by day 14, contrasting sharply with the slowest healing observed in the untreated group. Microscopically, on day 3, all burn wounds exhibited extensive necrotic tissue and diffuse inflammatory infiltration, consistent with the initial injury phase. By day 7, both hydrolate groups displayed regenerative changes, including fibroblast proliferation and re-epithelialisation. *S montana* L consistently demonstrated regenerative effects, leading to near-complete re-epithelialisation and minimal dermal inflammation by day 14.

Immunohistochemical assessment of IL-1β expression demonstrated distinct temporal patterns of inflammation across groups. On day 3, all animals exhibited a comparable inflammatory response characteristic of thermal injury. By day 7, the untreated group maintained a high proportion of IL-1β positive cells, whereas the *S montana* L group showed a peak decline, indicating an attenuation of the acute inflammatory phase. *Origanum vulgare* L and standard care groups also displayed a decline, although less pronounced. By day 14, IL-1β expression in the *S montana* L group

was minimal, suggesting near-complete resolution of inflammation, while persistent expression in the untreated group indicated progression toward chronic inflammation. The downregulation of IL-1β in the *S. montana* L group likely facilitated the transition to tissue regeneration and contributed to improved healing dynamics.

In addition, male New Zealand White rabbits were used in this study. The selection of a single sex aimed to minimise potential variability in physiological parameters and wound healing rates that could arise from hormonal fluctuations associated with oestrous cycles in female animals.[25] This approach provides a more stable baseline for assessing the direct effects of the hydrogel preparations. However, acknowledging the potential influence of sex hormones on wound healing, future studies should include female cohorts to ensure comprehensive understanding of the therapeutic potential across genders and enhance translational relevance.

Collectively, presented findings suggest a synergistic action of the bioactive compounds within *S montana* L and *O vulgare* L hydrogel forms. The observed reductions in pro-inflammatory cytokines, accelerated re-epithelialisation and restoration of the natural redox system paint a holistic picture of improved wound healing. While this study provides interesting evidence for the efficacy of these hydrolates, further molecular investigations are warranted to elucidate the precise mechanisms by which these compounds exert their effects, including their interaction with specific growth factors and signalling pathways involved in tissue repair. Additionally, optimising formulations and exploring broader applications represent important avenues for future research.

Conclusion

Presented findings establish that hydrolate-based gels, particularly the formulation derived from *Satureja montana* L and *Origanum vulgare* L are an effective therapeutic for accelerating the resolution of severe thermal burns. The specific hydrolate from *S montana* L demonstrated potent multifactorial activity, simultaneously suppressing inflammation, restoring redox homeostasis and promoting tissue regeneration, which collectively led to re-epithelialisation.

Ethics

The study was approved by the Bioethics Committee of the Institute of Translational Medicine and Biotechnology of Sechenov University (Protocol No 28, dated 21 October 2023). All manipulations were carried out in accordance with the International Guiding Principles for Biomedical Research Involving Animals (EEC, Strasbourg, 1985), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (EEC No 123, Strasbourg, 18.03.1986 (as amended on 22.06.1998)), the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) and ILAR (Institute for Laboratory Animal Research) guidelines for the care and use of laboratory animals, the Rules of Good Laboratory Practice and the Order of the Ministry of Health of the Russian Federation No 199n of 01.04.2016 "On approval of the rules of good laboratory practice".

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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