Trauma Monthly 2025; 30(4): 1546-1559

10.30491/tm.2025.536183.1857

Trauma Monthly

Research Article

The effects of Polyvinyl Alcohol–Chitosan Hydrogel Loaded with *Moringa Oleifera* Extract on Healing of Infected Burn Wounds in Rats Under High-Intensity Interval Training

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Received 2025-02-05; **Accepted** 2025-08-25; **Online Published** 2025-10-29

Abstract

Introduction: Burn injuries represent a critical medical challenge due to their prolonged healing time, susceptibility to infection, high treatment costs, and risk of systemic complications. Recent therapeutic approaches have focused on combining physical interventions and phytotherapeutic agents to enhance wound repair. This study aimed to investigate the synergistic effects of a polyvinyl alcohol–chitosan hydrogel infused with *Moringa oleifera* seed extract and high-intensity interval training (HIIT) on the healing of infected third-degree burn wounds in rats.

Methods: A hydrogel formulation based on polyvinyl alcohol and chitosan was synthesized incorporating *Moringa oleifera* extract. Male Wistar rats (n=72) were randomly assigned to four groups: T1 (HIIT alone), T2 (HIIT + hydrogel), T3 (hydrogel alone), and T4 (control). HIIT was administered for three weeks prior to wound induction. All animals received third-degree burn wounds followed by infection with *Staphylococcus aureus*. Wound healing was assessed both macroscopically and microscopically on days 7, 14, and 21 post-injuries.

Results: Macroscopic evaluation showed significantly greater wound contraction in T2 (combined HIIT and hydrogel) compared to other groups during the first and second weeks ($p < 0.05$). By the third week, although no significant difference was observed among the treated groups (T1–T3), all outperformed the control ($p > 0.05$). Histological analysis revealed enhanced angiogenesis, re-epithelialization, fibroplasia, collagen deposition, and reduced inflammatory infiltration in all treatment groups, with T2 consistently demonstrating the most pronounced improvements.

Conclusion: Both HIIT and topical application of *Moringa oleifera*-enriched chitosan hydrogel individually contributed to improved wound healing outcomes. However, their combination resulted in synergistic effects, promoting accelerated wound closure, enhanced tissue regeneration, and better modulation of inflammatory cell infiltrations. This synergy may be due to HIIT-induced systemic increases in growth factors and antioxidants, potentiating the local anti-inflammatory and pro-angiogenic effects of *Moringa oleifera* extract.

Keywords: Burn Wound Healing, *Moringa Oleifera*, Chitosan Hydrogel, High-Intensity Interval Training, Phytotherapeutic Biomaterial

Introduction

Wound healing is a tightly regulated biological response triggered when the skin's structural and functional integrity is compromised. This process unfolds through three overlapping but distinct phases: the inflammatory stage (0–3 days), the proliferative stage (2–12 days), and the remodeling or maturation phase (spanning 3 to 6 months).[1–3] During the initial phase, acute inflammation leads to the destruction of specialized cells and extracellular matrix, followed by the regeneration of new tissue to restore normal function. However, microbial contamination at the wound site often disrupts this repair cascade, significantly delaying recovery and affecting patient outcomes.[4] Effective wound healing, therefore, requires not only the restoration of tissue

architecture but also optimal vascular supply, oxygenation, and a moist environment to facilitate cell migration and proliferation.[5]

According to the World Health Organization, burn injuries remain a major global health burden, accounting for over 180,000 deaths annually.[6–8] These injuries, typically caused by extreme heat or corrosive chemicals can be classified into three types, with third-degree (full-thickness) burns being the most severe.[7,9,10] Such burns destroy all layers of the skin, causing extensive necrosis and damage to the extracellular matrix, with the most critical damage often located at the wound surface. [11,12] Although advances in burn management have considerably reduced mortality rates, allowing survival even in cases involving up to 100% total body surface area (TBSA) involvement, wound healing remains a complex challenge.[13–16]

Paradoxically, traditional wound dressings may sometimes introduce contaminants, particularly at the wound–exudate interface, or due to non-sterile materials.[17] In response to these limitations, hydrogel-based composite dressings have gained prominence. These dressings provide a moist healing environment, allow gas exchange, absorb excess exudate, and act as barriers to microbial invasion, all while being biocompatible, non-toxic, and environmentally friendly.[18] Commercially available dressings utilize various natural and synthetic polymers, such as alginic acid, chitosan, hyaluronic acid, cellulose, polyvinyl alcohol, and polycaprolactone, among others.[18]

Moringa oleifera, commonly known as the drumstick tree, is a medicinal plant native to the sub-Himalayan regions of northwest India. This species is renowned for its nutritional and therapeutic applications in Ayurvedic medicine and has been traditionally used to treat a wide range of conditions, including skin infections, parasitic infestations, tumors, and nervous disorders.[19,20] Modern studies confirm its antioxidant and antifungal effects, as well as its antimicrobial action against various human pathogens, which has even led to its inclusion in portable water purification systems.[21-23] Recognized by the WHO as a potential remedy for malnutrition, *M. oleifera* is rich in bioactive compounds that contribute to its therapeutic efficacy.[24,25] Phytochemical analysis of its seeds confirms the presence of essential constituents like flavonoids, saponins, and phenolics that support tissue regeneration and wound closure.[20,21,24,2] Notably, Rathi et al. [27] demonstrated the wound healing potential of aqueous extracts derived from the seeds and pulp of *M. oleifera* in an animal model.

Moreover, lifestyle factors such as exercise play a critical role in modulating immune function and wound repair. Among these, physical activity has been particularly highlighted for its regulatory effects on inflammatory mediators and the hypothalamic-pituitary-adrenal axis.[28] Exercise has also been associated with reduced psychological stress, which in turn, is linked to lower cortisol levels and improved immune responses.[29–31] High-intensity interval training (HIIT), a time-efficient exercise modality, has been shown to reduce pro-inflammatory cytokines and stress biomarkers over interventions spanning 10 to 12 weeks.[29,30] While the individual potential of hydrogel dressings and exercise is recognized, no study to date has investigated the potential synergistic effect of pre-conditioning with HIIT and the topical application of a *M. oleifera*-enriched hydrogel on the healing of severe, infected burn wounds. Therefore, the present study aimed to investigate the combined effects of a polyvinyl alcohol–chitosan hydrogel enriched with *Moringa oleifera* extract and HIIT on the healing of infected third-degree burn wounds in a rat model.

Methods

Extraction of *Moringa oleifera* Leaf Extract

Fresh *Moringa oleifera* leaves were collected, washed, and shade-dried at room temperature for 7–10 days. The dried leaves were then ground into a fine powder using a laboratory mill. For extraction, 100 grams of the powdered leaves were macerated in 500 mL of 70% ethanol at room temperature for 72 hours in a sealed, dark glass container. The mixture was intermittently stirred to enhance extraction. After maceration, the solution was filtered through sterile gauze followed by Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator under reduced pressure at 40–45°C to remove the solvent. The semi-solid extract was further dried in a hot air oven at 40°C to obtain a dry powder, which was stored in airtight, amber-colored containers at 4°C until further use.

Preparation of PVA-Chitosan Hydrogel Containing *Moringa oleifera* Extract

To prepare the hydrogel formulation, 10 g of polyvinyl alcohol (PVA) powder (with high degree of hydrolysis and medium molecular weight) was gradually dissolved in 100 mL of distilled water by continuous stirring at

80–90°C for approximately 1 hour until a clear and homogeneous solution was obtained. Simultaneously, a 1% (v/v) acetic acid solution was used to dissolve 1 g of chitosan powder (with a deacetylation degree of ~85% and medium molecular weight) in 100 mL, under magnetic stirring until a uniform chitosan solution was achieved. Subsequently, the chitosan solution was added dropwise to the PVA solution under sterile conditions, and the mixture was stirred continuously at room temperature for 1 hour to ensure homogeneity. The volume ratio of PVA to chitosan was maintained at 4:1 to optimize mechanical and biological properties of the hydrogel. Following the polymer blend preparation, 1 g of dried *M. oleifera* extract powder was added to the 100 mL polymer solution and stirred for an additional 30 minutes at room temperature to allow for uniform dispersion of the extract within the matrix. To induce gelation and crosslinking, the freeze–thaw cycling method was employed. The prepared solution was poured into sterile molds and subjected to freezing at −20°C for 24 hours, followed by thawing at room temperature for 4 hours. This freeze–thaw cycle was repeated three times to facilitate the formation of a stable three-dimensional hydrogel network. Finally, the hydrogels were removed from the molds and stored in sterile, airtight containers at 4°C until further use in subsequent experimental procedures.

Experimental animals

A total of 72 male Wistar rats, aged 2–3 months and weighing approximately 240 to 300 grams, were obtained from the Laboratory Animal Breeding and Maintenance Center of the Faculty of Medicine, Mashhad University of Medical Sciences. Upon arrival, the animals were housed in suitable cages in the Neurology Department animal facility at the School of Paramedical Sciences. To reduce transport-related stress and allow acclimatization to the new environment, the rats were kept for a period of 7 days under standard laboratory conditions, including natural room temperature (25°C), humidity 50 ± 2 proper ventilations, and a regular 12-hour light/dark cycle, with *ad libitum* access to water and standard laboratory chow (Javaneh Khorasan Co. Iran).

Following the acclimatization period, the rats were randomly assigned into four groups of 18 animals each cage. Group 1 (T1) was subjected to high-intensity interval training (HIIT) for three consecutive weeks. Group 2 (T2) received the same HIIT protocol along with topical application of the prepared hydrogel twice daily (every 12 hours). Group 3 (T3) was treated only with the hydrogel, without undergoing any exercise. Group 4 (T4) served as the control group and did not receive either exercise or hydrogel treatment.

High-Intensity Interval Training (HIIT) Protocol

In the present study, a structured high-intensity interval training (HIIT) program was implemented over a three-week period, consisting of three sessions per week. This protocol was designed based on the fundamental principles of HIIT and adapted from a previously validated methodology.[32] All training sessions were carried out on a motorized treadmill (Gene Iran Co.) and included three phases: warm-up, main training, and cool-down. Each session began with a 5-minute warm-up at 40% of the rats' maximum running speed and concluded with a 5-minute cool-down at the same intensity to ensure safe cardiovascular adaptation.

The central part of the training involved alternating 1-minute high-intensity running bouts with 1-minute active recovery periods, performed at 55% of the maximum running speed. The treadmill incline was maintained at 0% throughout all sessions to isolate the effects of speed-based interval training. To ensure progressive overload and adaptation, the intensity and volume of training were gradually increased over the three-week protocol. In the first week, rats performed five high-intensity intervals at 80% of their maximum speed, interspersed with five recovery intervals. In the second week, both the intensity and duration of the session were increased, with six intervals performed at 85% of maximum speed. Finally, during the third week, the number of intervals rose to seven, conducted at 90% of maximum speed. Recovery intervals remained consistent at 55% throughout the study.

As a result of this progression, the total duration of each training session increased from approximately 20 minutes in week one to 22 minutes in week two, and 24 minutes in the final week. This structured HIIT approach aimed to elicit metabolic and physiological adaptations relevant to tissue repair, immune modulation, and overall enhancement of wound healing capacity in the experimental model.

Burn Wound Induction and Infection Procedure

To induce third-degree burn wounds, animals were initially anesthetized via intramuscular injection of ketamine 10% (70 mg/kg) and xylazine 2% (5 mg/kg) administered into the posterior thigh. Anesthesia was maintained using 4% isoflurane for induction and 2.5% for maintenance, delivered in oxygen at a flow rate of

0.3 L/min. The dorsal surface of each rat, from below the neck to the mid-back, was shaved bilaterally.

Burn injuries were created by applying a copper rod (1 cm diameter), preheated in boiling water (100°C) for 5 minutes, to two distinct dorsal sites for 20 seconds each without exerting pressure. This resulted in full-thickness (third-degree) thermal burns.

To establish localized infection, 30 minutes after burn induction, 100 µL of a freshly cultured suspension of *Staphylococcus aureus* (ATCC 43300) prepared in Tryptic Soy Broth at 37°C for 18-24 hours and standardized to a concentration of 5×10^7 CFU/mL was topically applied to each wound. Each wound was covered with a sterile gauze pad secured in place using surgical skin clips. This method led to localized infection within 24 hours.[33]

During the procedure, core body temperature was maintained between 36–37°C using a homeothermic blanket system (Harvard Apparatus). Fluid resuscitation was performed via intraperitoneal injection of 1 mL Ringer's lactate solution. After 24 hours, gauze was removed from all animals, treatment protocols were initiated, and rats were returned to individual cages and monitored daily.

Throughout the experimental period, all animals in all groups were closely monitored for general health status, signs of wound infection, hydration levels, and food intake. These parameters were specifically assessed during the first 72 hours following wound induction to ensure animal welfare and detect any early signs of systemic or local complications. In groups receiving hydrogel treatment (T2 and T3), the formulated hydrogel was applied topically to the wound site twice daily (every 12 hours) under aseptic conditions throughout the experimental period.

Macroscopic Wound Assessment

On days 7, 14, and 21 following burn induction, standardized digital photographs of the wound sites were captured using a fixed camera setup under consistent lighting and distance conditions. These images were subsequently analyzed using ImageJ software, allowing for precise and reproducible measurement of the wound surface area (in cm^2) after proper calibration. To objectively assess wound healing over time, the percentage of wound contraction for each animal was calculated at the specified intervals using the following formula:

Wound Contraction (%) = ([Initial Wound Area – Wound Area on Specific Day] / Initial Wound Area) × 100

This quantitative approach provided a reliable metric for evaluating the rate and extent of wound closure across the experimental groups.

Histopathological Evaluation

Histopathological evaluation of skin tissue was carried out on days 7, 14, and 21 following burn induction. On each of these time points, six rats were randomly selected from each experimental group and humanely euthanized using sodium thiopental. Full-thickness skin biopsies approximately 5 mm in diameter were excised from the wound site of each animal and immediately fixed in 10% neutral-buffered formalin. The fixed tissues were processed using standard paraffin embedding procedures, and 5 µm-thick sections were prepared using a microtome. These sections were stained with hematoxylin and eosin (H&E) following conventional histological staining protocols. Blinded microscopic analysis was performed by an experienced pathologist using a light microscope. For each specimen, at least five random microscopic fields were examined, and key histopathological features including angiogenesis, epidermal regeneration, inflammatory cell infiltration, collagen fiber density, and fibroblast proliferation were evaluated and scored qualitatively as absent, mild, moderate, or severe.

Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics software, version 27 (Armonk, NY, USA). To compare the mean values across different experimental groups, a two-way ANOVA was employed. All quantitative data were expressed as mean ± standard deviation (SD). A p-value of less than 0.05 ($P < 0.05$) was considered indicative of statistical significance.

Results

Wound Contraction

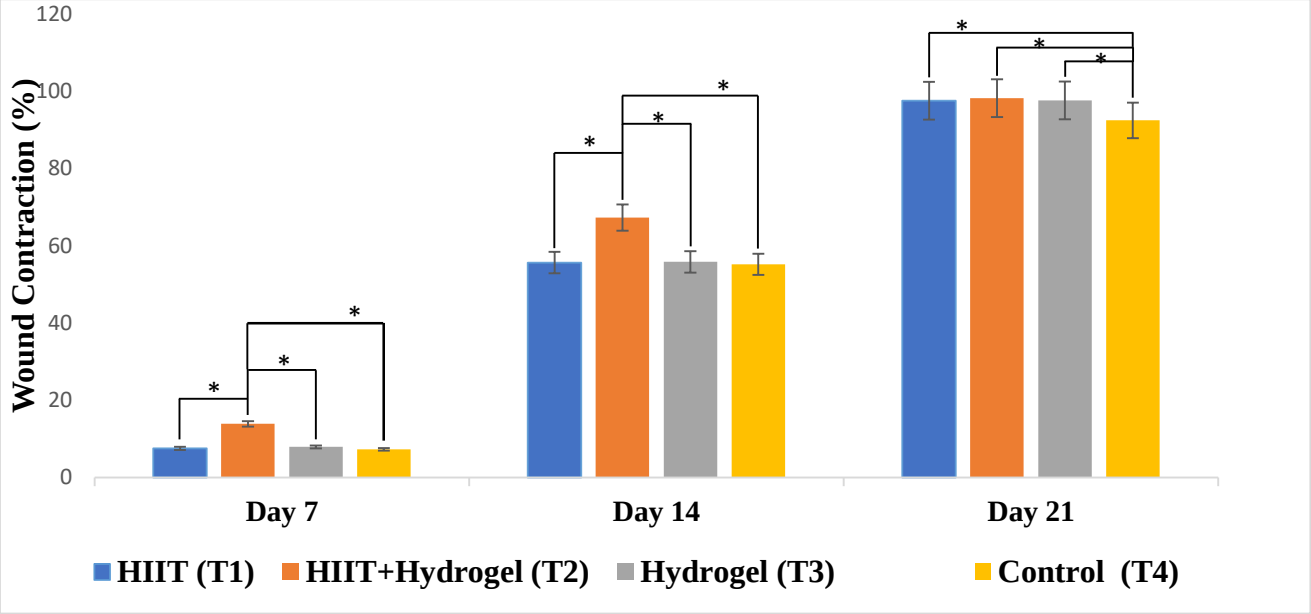
Wound contraction percentages for each group were assessed on days 7, 14, and 21 post-burn induction using ImageJ software and the standard formula for wound area reduction (Table 1, Graph 1). On days 7 and 14, the group receiving both the hydrogel treatment and high-intensity interval training (T2) exhibited a significantly greater percentage of wound contraction compared to all other groups ($p < 0.05$). By day 21, although no statistically significant differences were

observed among the T1 (HIIT only), T2 (HIIT + hydrogel), and T3 (hydrogel only) groups ($p > 0.05$), each of these treatment groups demonstrated a markedly higher degree of wound contraction relative to the control group (T4) ($p < 0.05$).

Table 1: Mean ± standard deviation of wound contraction percentage (%) in Wistar rats across different treatment groups during three weeks of study

Days / Groups	T1	T2	T3	T4
Day 7	7.51 ± 1.62^{b}	13.87 ± 1.43^{a}	7.89 ± 0.52^{b}	7.25 ± 0.63^{b}
Day 14	55.60 ± 1.12^{b}	67.26 ± 2.54^{a}	55.81 ± 2.38^{b}	55.15 ± 3.75^{b}
Day 21	97.53 ± 1.24^{b}	98.17 ± 1.75^{b}	97.62 ± 0.74^{b}	92.43 ± 1.01^{a}

^{ab}Different superscript letters mean in each row showed significance level at $p \leq 0.05$. T1: High-intensity interval training (HIIT), T2: HIIT plus hydrogel. T3: Only hydrogel, T4: Control group.



Graph 1: Wound contraction percentage (%) in Wistar rats across treatment groups (T1-T4) over 21 days. Values marked with (*) indicate significant differences between the groups ($p < 0.05$)

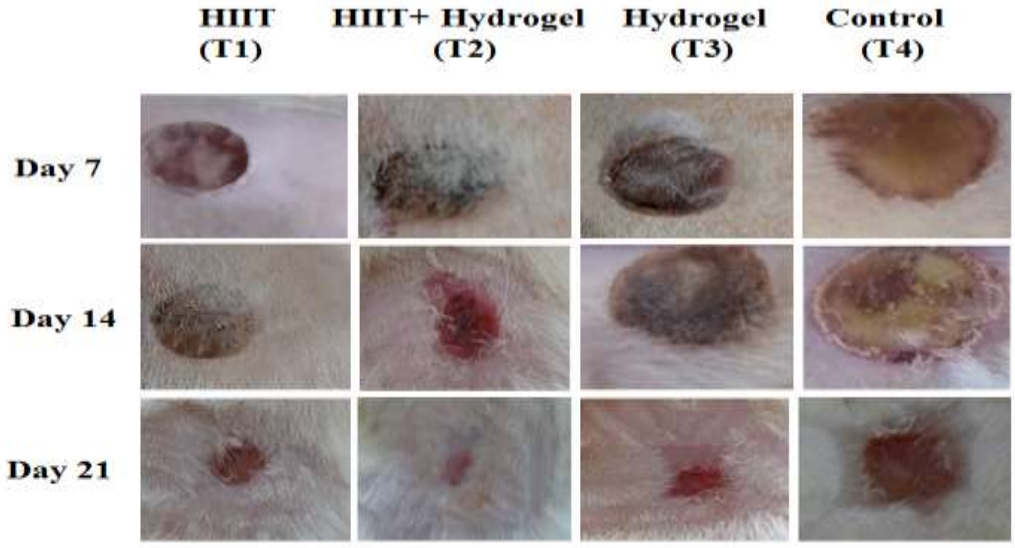


Figure 1: Macroscopic images of wound in Wistar rats across treatment groups (T1-T4) on each day 7,14 and 21

Histopathological Investigation

Based on the histopathological evaluations, wound healing parameters including re-epithelialization, inflammatory cell infiltration, fibroplasia, collagen deposition, and angiogenesis were assessed and scored individually across all groups on days 7, 14, and 21 post-burn induction. With respect to re-epithelialization, moderate re-epithelialization was observed on day 7 in both the T2 group (chitosan hydrogel containing *M. oleifera* extract combined with HIIT) and the T3 group (hydrogel only) (Figures 3 and 4). In contrast, the T1 group (HIIT only) and the control group (T4) demonstrated only mild re-epithelialization at this time point (Figures 2 and 5). By day 14, the extent of re-epithelialization had progressed markedly in groups T2 and T3, both of which exhibited severe re-epithelialization (Figures 3 and 4), whereas groups T1 and T4 showed moderate re-epithelialization (Figures 2 and 5). On day 21, all three intervention groups (T1, T2, and T3) showed severe re-epithelialization, indicating advanced epidermal regeneration, while the control group remained at a moderate level of re-epithelialization (Figures 2, 4, and 5).

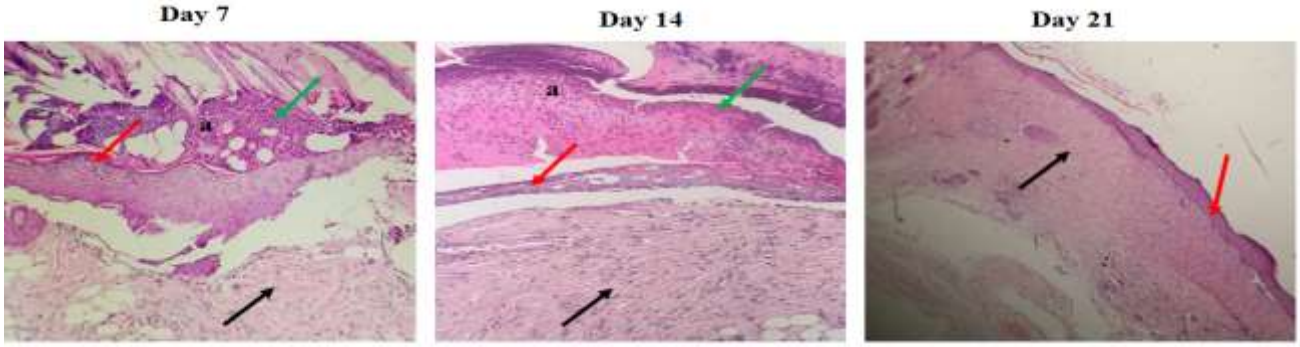


Figure 2: Histopathological assessment of Group T1 (HIIT only) on days 7, 14, and 21 post-burn injury using hematoxylin and eosin (H&E) staining. On day 7, the tissue exhibited a scab composed of necrotic debris and degenerated polymorphonuclear leukocytes (PMNLs). Mild re-epithelialization (red arrow) was evident, along with granulation tissue containing a moderate proliferation of fibroblasts (black arrow), a mild infiltration of inflammatory cells (a), a moderate amount of collagen deposition, and moderate neovascularization beneath the regenerating epithelium. Residual scab tissue (green arrow) was still present (H&E, ×100). By day 14, the tissue maintained a necrotic scab and degenerated PMNLs, while re-epithelialization progressed to a moderate level (red arrow). The granulation tissue revealed moderate fibroblast activity (black arrow), a mild inflammatory cell infiltration (a), a severe degree of collagen deposition, and moderate angiogenesis beneath the forming epithelium. Residual scab material (green arrow) was also observed (H&E, ×100). On day 21, the scab was mildly formed and composed of necrotic debris (green arrow). There was severe and nearly complete re-epithelialization (red arrow). The underlying dermis (black arrow) demonstrated moderate fibroblast proliferation, mild inflammatory cell infiltration (predominantly macrophages), moderate collagen content, and moderate neovascularization beneath the mature epithelium (H&E, ×40).

In terms of the time required for complete epithelial coverage of the wound surface, groups T2 (hydrogel with *M. oleifera* extract + HIIT) and T3 (hydrogel only) demonstrated near-complete re-epithelialization as early as day 7 (Figures 3 and 4). By day 14, both groups exhibited fully matured and stratified epithelial layers, indicating complete re-epithelialization and restoration of epidermal integrity (Figures 2 and 3). In contrast, group T1 (HIIT only) reached this stage by day 21, showing a delayed but eventually complete epithelial regeneration (Figure 2). The control group (T4), however, failed to achieve full re-epithelialization even by day 21, with only partial epithelial formation observed, indicating incomplete wound closure and delayed healing (Figure 5).

Regarding the presence of inflammatory cells, histopathological assessments on days 7 and 21 revealed that groups T1, T2, and T3 exhibited only a mild level of inflammatory cell infiltration, predominantly composed of macrophages (Figures 2, 3, and 4). In contrast, the control group displayed a severe inflammatory cell infiltration on day 7, which subsided to a moderate level by day 21 (Figure 5). Notably, in group T2, inflammatory cell presence slightly increased on day 14 (Figure 3) but remained mild by day 21, indicating effective inflammation resolution. In the control group, the number of inflammatory cells showed a gradual decrease from day 14 through day 21.

In terms of fibroplasia, on day 7, groups T1 and T2 demonstrated moderate fibroblast proliferation (Figures 2 and 3), while only mild fibroplasia was evident in group T3 and the control group (Figures 4 and 5). By day 14, severe fibroplasia was observed in T2 and T3 (Figures 3 and 4), whereas T1 and the control group showed a moderate increase in fibroblast activity (Figures 2 and 5). On day 21, fibroplasia remained moderate in T1, T3, and the control group (Figures 2, 4, and 5), while group T2 maintained a severe level of fibroblast proliferation, highlighting its enhanced regenerative capacity (Figure 3).

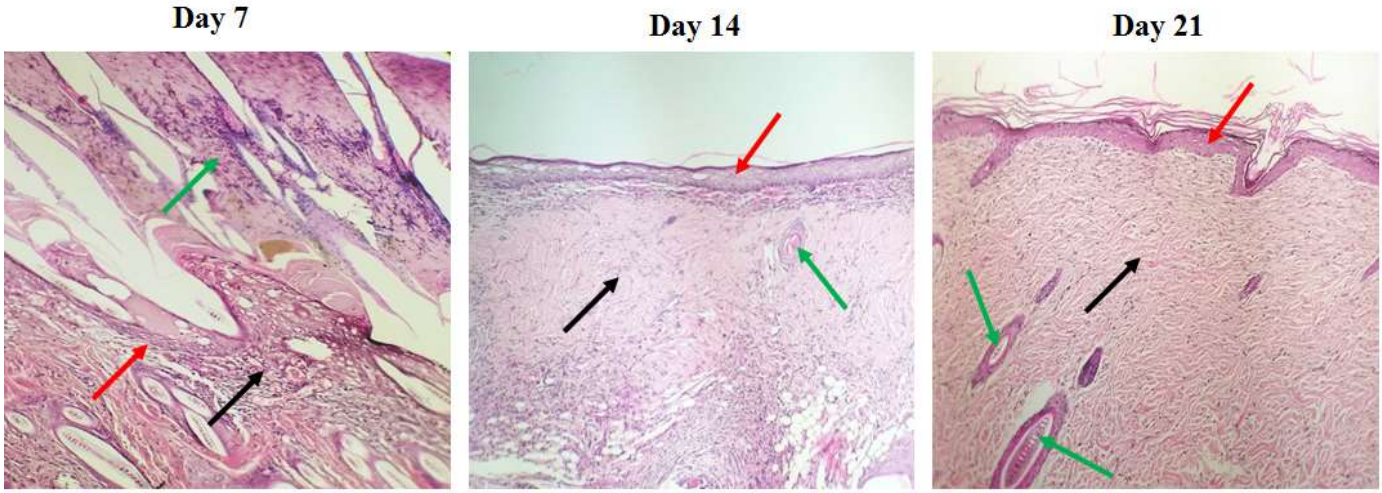


Figure 3: Histopathological examination of Group T2 (receiving both HIIT and chitosan hydrogel with *Moringa oleifera* extract) under light microscopy. On day 7, moderate but complete re-epithelialization was observed, with the newly formed epithelial tissue fully covering the wound surface (red arrow). The underlying dermis showed areas of tissue loss and necrosis, replaced by granulation tissue (black arrow) exhibiting a mild presence of inflammatory cells, moderate fibroblast proliferation, moderate collagen deposition, and moderate neovascularization (green arrow: scab) (H&E, ×100). On day 14, the epithelial layer continued to cover 100% of the wound area (red arrow). The dermal layer consisted of granulation tissue (black arrow) with a moderate level of inflammatory cells, alongside severe fibroblast proliferation, intense collagen deposition, and marked formation of new blood vessels (green arrow) (H&E, ×100). By day 21, the epithelial coverage of the wound area was complete and mature (red arrow), with the underlying dermis (black arrow: granulation tissue) showing minimal inflammatory infiltration, severe fibroblast activity, dense collagen accumulation, and extensive neovascularization (green arrow) (H&E, ×100).

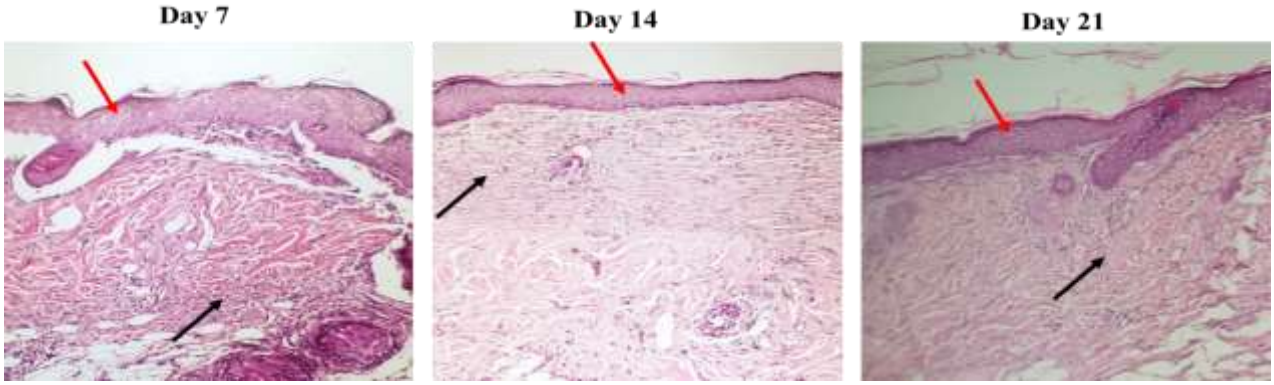


Figure 4: Histopathological analysis of tissue samples from group T3 (treated with hydrogel only), examined under light microscopy. On day 7, Moderate yet complete re-epithelialization was observed, with the newly formed epithelial layer covering the entire wound area. The underlying dermis showed signs of necrosis and was replaced by granulation tissue. Mild infiltration of inflammatory cells (primarily macrophages), mild fibroblast proliferation, moderate collagen deposition, and mild neovascularization were evident (H&E, ×100). (Red arrow: epithelialization; Black arrow: granulation tissue); on day 14: The epithelium remained fully regenerated, covering the full wound surface. The dermal layer demonstrated severe fibroblast proliferation, severe collagen presence, severe neovascularization, and mild inflammatory cell infiltration (mainly macrophages), indicating active tissue remodeling (H&E, ×100) (Red arrow: epithelialization; Black arrow: granulation tissue); on day 21, complete epithelial coverage persisted across the wound surface. The underlying dermis displayed moderate fibroblast activity, severe collagen deposition, severe formation of new blood vessels, and a mild presence of inflammatory cells (H&E, ×100). (Red arrow: epithelialization; Black arrow: granulation tissue)

Regarding collagen deposition, on day 7, all four groups exhibited moderate levels of collagen presence (Figures 2–5). By day 14, groups T1, T2, and T3 showed a marked increase, displaying severe collagen deposition (Figures 2–5), whereas the control group remained at a moderate level (Figure 4). On day 21, severe collagen deposition persisted in groups T2 and T3 (Figures 3 and 4), while groups T1 and the control group continued to show moderate collagen presence (Figures 2 and 5).

As for angiogenesis, mild neovascularization was noted in groups T3 and the control group on day 7 (Figures 4 and 5). The control group maintained this mild level on day 14 and showed a moderate increase by day 21 (Figure 5). Conversely, groups T2 and T3 showed enhanced angiogenic activity, progressing to severe levels by days 14 and 21 (Figures 3 and 4). On day 7, T2 exhibited moderate and T3 mild angiogenesis, respectively. Group T1 consistently demonstrated moderate angiogenesis throughout the entire study period (Figure 2).

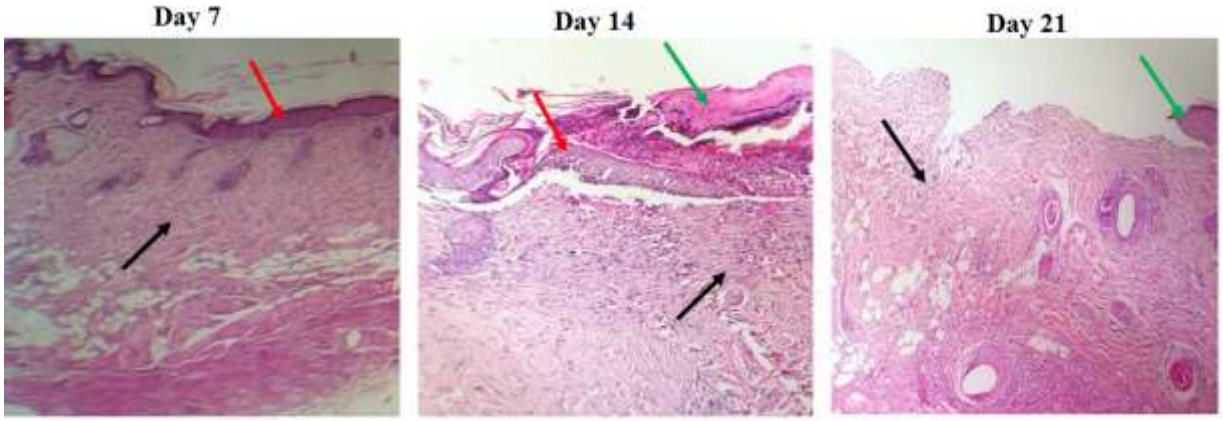


Figure 5: Histopathological examination with light microscopy of group T4 (Control Group). On day 7, the tissue section revealed a scab composed of necrotic debris and degenerated polymorphonuclear cells (PMNLs). Mild re-epithelialization was observed (Red arrow), along with granulation tissue characterized by mild fibroblast proliferation (Black arrow), severe infiltration of inflammatory cells, moderate collagen deposition, and mild formation of new blood vessels beneath the regenerating epithelium. Residual scab tissue was also evident (H&E, ×100). On day 14, the wound area continued to exhibit a necrotic scab with degenerated PMNLs (Green arrow). Re-epithelialization had progressed to a moderate level (Red arrow). The granulation tissue showed moderate fibroblast proliferation (Black arrow), mild presence of inflammatory cells, moderate collagen deposition, and mild neovascularization. On day 21, histological analysis showed a persistent scab formed of necrotic debris and degenerated PMNLs (Green arrow). Re-epithelialization remained moderate and incomplete (Red arrow). The dermal layer exhibited moderate fibroblast proliferation (Black arrow), moderate presence of inflammatory cells, collagen, and moderately developed new blood vessels under the forming epithelium (H&E, ×100).

Discussion

Wound healing is a dynamic and multifaceted biological process involving a complex interplay between inflammatory cell infiltrations, cellular proliferation, angiogenesis, and tissue remodeling. Various intrinsic and extrinsic factors can influence the rate and quality of tissue repair, including infection, local perfusion, immune status, and therapeutic interventions.[34,35] In recent years, alternative approaches such as physical exercise, phytotherapeutics, and biomaterial-based dressings have gained attention as potential modulators of wound healing.[36] The present study aimed to explore the combined and individual effects of high-intensity interval training (HIIT) and a plant-based hydrogel derived from *M. oleifera* on the healing of infected burn wounds in a rat model. The findings provide valuable insights into how these interventions may influence different phases of wound repair, particularly in the context of infection-compromised healing environments.

Recent studies have clearly demonstrated that physical activity can enhance the process of cutaneous wound healing.[37,38] In the present study, rats subjected to high-intensity interval training (HIIT) alone (T1 group) showed significant improvements in several wound healing parameters compared to the control group. The percentage of wound contraction on days 7 and 14 was significantly higher, and histopathological analysis revealed more organized tissue architecture in this group. These findings align with those of Emery et al.[37], who reported that older adults engaged in moderate-intensity aerobic exercise for three months exhibited accelerated wound healing compared to sedentary controls. They attributed this to improved neuroendocrine responses and proposed further evaluation of pro-inflammatory cytokine dynamics at the wound site. Similarly, another study showed that aged mice trained at 70% VO2max displayed reduced wound size in the early healing phase (up to six days post-wounding), along with reduced levels of TNF-α, KC, and MCP-1 in the wound environment.[38] These anti-inflammatory effects, observed particularly in the early phase, may be further enhanced with prolonged exercise regimens, as moderate physical conditioning is known to reduce inflammation through chronic adaptations.[39] The present study similarly observed a reduction in inflammatory cells, particularly neutrophils, in the HIIT group compared to the control group during the first week. This suggests a shortened inflammatory phase potentially driven by systemic cytokine balance induced by exercise, as previously proposed.[40] Nonetheless, it should be noted that in certain contexts, high-intensity exercise might provoke inflammatory cell infiltrations. Gokhale et al.[41] demonstrated that strenuous exercise elevated serum levels of TNF-α and IL-6, which may exacerbate inflammation. Furthermore, increased lactate production under anaerobic conditions during high-intensity exercise could have contributed to enhanced fibroblast proliferation and angiogenesis, particularly in the first week. This is consistent with prior studies reporting that lactate and vascular endothelial growth factor (VEGF) are involved in stimulating fibroplasia and neovascularization.[42,43] Indeed, our data showed that the T1 group exhibited higher levels of fibroplasia and angiogenesis during the first week than the control group.

Exercise-induced collagen synthesis is another important aspect of connective tissue remodeling. Previous research has demonstrated increased collagen turnover in trained animals, including better collagen fiber organization.[44-47] In the present study, collagen density in the T1 group was higher than that of the control group by the second week, suggesting an upregulation of collagen synthesis pathways in response to physical training. Thus, high-intensity interval training (HIIT) appears to promote wound healing in infected burn wounds by modulating the inflammatory cell infiltration, stimulating fibroblast activity, enhancing angiogenesis, and increasing collagen deposition. These findings are consistent with existing literature and support the use of HIIT as a potential adjunct therapy to improve the quality and speed of wound healing.

Hydrogels have emerged as promising wound dressing materials due to their biocompatibility, moisture-retentive properties, and capacity for sustained release of therapeutic agents. In the present study, the hydrogel formulated with *M. oleifera* extract (T3 group) significantly improved wound healing outcomes compared to the control group. Enhanced wound contraction, angiogenesis, and fibroplasia were evident, particularly during the second week of healing.

These results align with prior studies that have

demonstrated the wound healing efficacy of *M. oleifera* in various forms. Kumar et al.[48] showed that the aqueous leaf extract of *M. oleifera* accelerated wound closure in Swiss albino rats and enhanced tensile strength in incision wounds (507.5 g on day 10). Rathi et al.[27] reported that systemically administered aqueous extract from seeds and pulp of *M. oleifera* promoted healing across excision, incision, and dead space wound models. Similarly, a study conducted in Algeria highlighted the anti-inflammatory and wound healing potential of seed-derived polyphenol and saponin extracts, with saponins exhibiting significantly greater efficacy (28.16%) compared to polyphenols (23.61%) and outperforming the reference drug Madecassol®. [49] Other findings reinforce the bioactivity of *M. oleifera* seed extracts in topical formulations. Coker et al.[50] showed that 10% ethyl acetate extract of dried leaves and seeds in ointment form significantly enhanced healing in excision and incision models. In another study, the biopolymeric components of *M. oleifera* seeds and *Acacia arabica* demonstrated antibacterial and hemostatic activity, including shortening of prothrombin and partial thromboplastin times, making them promising for infection-prone wounds.[51] Furthermore, a novel MSP/PVA hydrogel composed of *M. oleifera* seed polysaccharide and polyvinyl alcohol exhibited hemocompatibility, bacterial impermeability, and antioxidant activity, offering a multifunctional approach to chronic wound management.[52] The improved outcomes observed in our study may also be attributed to the hydrogel formulation itself. Hydrogels offer the advantage of uniform spreading, sustained release, and enhanced skin permeability, thereby improving the bioavailability of phytochemicals at the wound site. Notably, while Momoh et al.[53] reported slower healing with aqueous leaf extract, our formulation showed faster wound contraction, likely due to improved topical delivery and synergistic effects of multiple bioactive compounds within the hydrogel matrix. In same line with the results of the present study, a recent study demonstrated that HIIT prior to wounding and the use of chitosan hydrogel containing Burdock root extract as a wound dressing, each individually, and positively influenced wound contraction and improved pathological wound healing factors. Additionally, the combined approach of HIIT and the use of this hydrogel can enhance the effects of each one on wound healing and rehabilitation.[54] In addition, another study revealed that alginate sulfate hydrogels-Platelet-derived growth factor (PDGF-BB) were faster in wound healing than non-sulfate alginate hydrogels-PDGF-BB.[55] Furthermore, Babavalian et al.[56] showed that treatment with peptide-containing hydrogel showed wound healing comparable to the positive control and demonstrated significant antibacterial activity against *S. aureus* wound infections in mice.

Altogether, these findings underscore the therapeutic potential of *M. oleifera* when delivered via hydrogel. Its phytoconstituents, including flavonoids, tannins, and saponins, likely contributed to the anti-inflammatory, antioxidant, and antimicrobial effects observed, all of which are critical to optimal wound healing. The incorporation of such bioactive compounds into hydrogel matrices may offer a safe, natural, and cost-effective strategy for managing infected or chronic wounds.

Infection is a major impediment to wound healing and can drastically alter the normal repair timeline.[33] Open wounds are highly susceptible to colonization by microbial pathogens due to the loss of skin barrier integrity and the dynamic, nutrient-rich environment created by wound exudates.[11] The initial microbial colonizers are often *Streptococcus* spp. and *Staphylococcus aureus*, which are later followed by opportunistic pathogens such as *Escherichia coli* and *Pseudomonas aeruginosa*, especially in wounds that remain untreated or inadequately managed.[33] These infections increase inflammation, hinder cellular proliferation, and contribute to prolonged or even chronic wound states.[7,8] Thus, effective antimicrobial management is a cornerstone in promoting successful wound healing and tissue restoration.

In the current study, the control group (T4) which received no treatment demonstrated the poorest wound healing outcomes during three weeks of the study. Histopathological evaluation revealed severe inflammatory infiltration, reduced neovascularization, delayed re-epithelialization, and poorly organized collagen fibers. These results are consistent with the expected progression of infected wounds left untreated, where bacterial proliferation exacerbates tissue degradation and inflammatory processes. The contrast between this group and the treated groups underscores the significant impact of microbial control on healing progression. The improved healing in groups treated with *M. oleifera* hydrogel may be attributed not only to

the hydrogel's physical properties such as moisture retention and controlled drug release but also to the intrinsic antimicrobial and antioxidant activities of *M. oleifera* seeds. Several studies have validated the antimicrobial potential of *M. oleifera*, particularly due to its phytochemical constituents including terpenoids, terpenes, glycosides, flavonoids, phenols, alkaloids, saponins, and tannins.[22,57,58] Flavonoids are especially noted for their ability to form complexes with extracellular proteins and bacterial cell walls, leading to microbial inhibition.[59] Moreover, prior studies have shown that *M. oleifera* seed extracts demonstrate broad-spectrum antibacterial effects. For instance, Coker et al.[50] reported significant activities of ethanolic and ethyl acetate extracts of *M. oleifera* seeds and leaves against wound-related pathogens. However, some resistance was observed in earlier research; pathogens such as *S. aureus* and *P. aeruginosa* were resistant to ethanolic extracts except in cases of *E. coli*, *P. vulgaris*, and *S. typhi*.[60]

Furthermore, the necessity for antimicrobial intervention is further supported by the known correlation between microbial load and delayed wound healing. Without proper microbial control, inflammatory mediators persist at elevated levels, neutrophil infiltration becomes excessive, and fibroblast activity is impaired all leading to disorganized collagen deposition and scar formation. The results in our control group provide a clear illustration of these mechanisms in action. Thus, the findings of this study highlight that an effective wound healing agent must address both tissue regeneration and microbial inhibition. The *M. oleifera*-based hydrogel, by virtue of its phytoconstituents and delivery system, offers a dual-function approach that not only accelerates tissue repair but also suppresses microbial colonization both of which are essential for restoring skin integrity and preventing chronic wound development.

This study has several limitations that should be acknowledged. Firstly, the histopathological analysis was qualitative and subjective. Secondly, the present study did not measure key mechanistic data, such as bacterial load to confirm infection control or cytokine levels to substantiate the anti-inflammatory effects. These factors should be considered when interpreting the results.

Conclusion

The present study evaluated the synergistic effects of high-intensity interval training (HIIT) and a chitosan-based hydrogel containing *M. oleifera* seed extract on the healing of traumatic burn wounds in a rat model. The results demonstrated that this combined intervention significantly enhanced the overall wound healing process when compared to untreated controls and groups receiving individual treatments. Improvements were evident in several key histological and physiological parameters, including accelerated wound contraction, enhanced re-epithelialization, increased fibroblast proliferation, organized collagen fiber deposition, and robust angiogenesis. Moreover, this approach appeared to modulate the local inflammatory cell infiltration effectively, reducing excessive infiltration while maintaining a conducive environment for tissue repair. The antimicrobial and antioxidant properties of *M. oleifera* seed extract, together with the moisture-retaining and bioactive delivery capabilities of the chitosan hydrogel, likely played a critical role in promoting tissue regeneration. Simultaneously, the systemic physiological benefits of HIIT, including improved circulation, immune regulation, and cytokine balance, may have further supported the reparative process. Collectively, the findings suggest that integrating phytotherapeutic hydrogel treatment with structured physical activity can serve as a promising therapeutic strategy for managing infected or delayed-healing wounds. Future studies are warranted to explore the molecular mechanisms underlying this synergistic effect and to assess its translational potential in clinical wound management.

Acknowledgments

The authors would like to express their gratitude to Dr. Sajad SahabNegah from the Department of Neuroscience, School of Medicine, Mashhad University of Medical Sciences, Iran for his valuable support and contributions.

Conflict of Interest Disclosures

The authors declare that they have no competing interests.

Funding Sources

The authors declared that this study received financial support from Baqiyatallah University of Medical

Sciences, Tehran, Iran.

Authors' Contributions

Pouria Ahmadi Simab conducted the practical procedure and performed sampling. Hamid Babavalian and Abolfazl Shakibaee designed the study and checked the full version of the article. Zahra Sobhani and Fatemeh Shakeri participated in the practical procedure. Fathollah Ahmadpour and Hadi Khoshmohabbat contributed to the revision of the draft of the manuscript. All authors check the final proof of the article and the statistical results.

Ethical Statement

The NIH"Guide for the Care and Use of Laboratory Animals" (NIH publication No. 80-23, revised 2011) and the professional governmental guidelines, in compliance with the Institutional Animal Care and Use Committee (IACUC) at Baqiyatallah University of Medical Sciences, Tehran, Iran, had been observed in all experiments (IR.BMSU.AEC.1401.014).

Declaration of Generative AI and AI-assisted technologies

We declare that no generative artificial intelligence (AI) or AI-assisted technologies were used in the preparation, writing, data analysis, or editing of this manuscript. All parts of this work were conducted and written entirely by the authors.

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