



Effect of Hydrogel Containing Herbal Extract of *Arctium lappa* root on Traumatic Burn Wound Healing in Rat under HIIT Exercise

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Abstract

Introduction: Traumas have always been one of the most significant types of injuries to the body, among which burn wounds are notable. The recent study was designed to investigate the characteristics of chitosan hydrogel and Burdock root extract, along with the effect of high-intensity interval training (HIIT) on burn wounds.

Methods: chitosan hydrogel containing Burdock root extract was designed as a wound dressing. Additionally, a HIIT regimen was designed and implemented for the groups three weeks prior to the start of the study. Seventy-two male Wistar rats were randomly divided into four equal groups. All groups were evaluated on days 7th, 14th, and 21st days after wound induction based on macroscopic and microscopic parameters.

Results: The results of macroscopic evaluation indicated that the wound contraction percentage in the first and second weeks showed a statistically significant difference in the group receiving HIIT and hydrogel, compared to other groups. In the third week, although there was no difference between the groups received only HIIT, H2, and H3 which only received hydrogel, all three groups demonstrated a statistically significant difference compared to the control group. Regarding the microscopic evaluation, all three groups performed better than the control group in terms of angiogenesis, re-epithelialization, inflammatory cells, collagen deposition, and fibroplasia, with slight differences among them. Among these groups, H2 showed superior performance in all parameters compared to other groups.

Conclusion: The 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.

Keywords: Traumas, Wound Dressing, HIIT, Chitosan, Burdock.

Introduction

Skin is the largest organ of the body and performs many vital functions, including protection against environmental threats (physical, chemical, and microorganisms). It also prevents excessive water loss from the body and plays a role in regulating body

temperature. Various traumas can compromise the integrity of the skin. Among the, burn wounds are the most significant, which can cause substantial financial and personal harm. Treating burns has always been a noteworthy and challenging issue in human medicine,

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and various methods have been used to address it. Generally, wound healing occurs in four phases common to all wound types, differing only in the duration of each phase. The first phase is hemostasis or coagulation phase, in which the damaged blood vessels form blood clot, which prevents blood loss from the site of damage. Platelet plug, consisting of fibrin, fibronectin, fibronectin, and thrombospondin, is formed to prevent bleeding. The platelet plug also shields the wound against bacterial invasion, while providing a scaffold for the immune cells and cytokines to guide the early repair. The second phase is the inflammatory phase, during which vascular permeability increases, and plasma leakage occurs. Neutrophils and monocytes, which are guided by a cascade of inflammatory mediators, are the first migrating cells to reach the site of inflammation. Following this, the inflammatory phase involves phagocytosis and the removal of cellular debris from the wound site. The next phase is the proliferation phase, where the wound is filled with new connective tissue called granulation tissue. In this stage, re-epithelialization, angiogenesis, fibroblast production, and collagen synthesis occur, leading to skin regeneration. The final phase is the maturation and remodeling phase, where extracellular matrix fibers and structures reorganize, shaping the scar tissue. Fibroblasts and collagen fibers mature and reshape, and ultimately, new skin forms at the wound site.¹

Arctium lappa, commonly known as burdock, is a perennial herb belonging to the family Asteraceae. It stores most of its nutrients during the first year of growth. This plant, found globally, has a long history of cultivation as a vegetable in Asia. Burdock is traditionally used to treat ailments such as sore throat, skin infections like rashes and boils, and various other skin problems. The dried root of one-year-old burdock is primarily used for therapeutic purposes, although extracts from different parts of the plant have long been considered beneficial for health. The biological activities and pharmacological functions associated with *Arctium* species include anti-inflammatory, anticancer, antidiabetic, antimicrobial, and antiviral activities. These properties make burdock a subject of interest in both traditional and modern medicine for its potential health benefits. Recently, many studies indicated the potential property of Burdock in wound healing process, as it reduces inflammation, and minimizes the possibility of infection at the wound site.² Additionally, research has demonstrated that burdock extract possesses antioxidant properties that

can act against free radicals and oxidative stress, thereby contributing to the wound healing process.³ Furthermore, burdock extract influences the stimulation and proliferation of fibroblasts, which can significantly impact wound repair and regeneration.⁴

An ideal skin wound dressing should fulfill several critical requirements, including sufficient mechanical strength, effective moisture retention, appropriate surface microstructure, and excellent tissue compatibility. Various biomedical materials can serve as wound dressings, such as fibrous membranes, polymer and polysaccharide scaffolds, nanoparticles, and hydrogels. Among these, hydrogel dressings have garnered significant attention due to their tunable physicochemical properties that closely resemble the extracellular matrix (ECM), their ability to modulate fluid balance, and their capability to accelerate wound healing. Natural polysaccharides like chitosan (CS) have become increasingly prominent owing to their high hydrophilicity and exceptional biocompatibility. Chitosan, in particular, possesses hemostatic and antibacterial properties, along with excellent cytocompatibility, addressing two major challenges in the initial stages of wound healing. Moreover, chitosan's anti-inflammatory properties make it an ideal candidate for the inflammation phase of wound repair. Additionally, chitosan can induce local macrophage proliferation and stimulate ECM remodeling, thereby promoting early wound healing.⁵ Thus, the primary biochemical effects of chitosan include the activation of fibroblasts, production of cytokines, migration of giant cells, and stimulation of type IV collagen synthesis. These actions lead to rapid infection clearance, swift wound debridement, effective suppression of inflammation, minimization of scarring, and promotion of angiogenesis, as well as the growth of granulation tissue and epithelial cells.⁶ Also, chitosan, a polyatomic polysaccharide derived from chitin, has gel-forming properties, which makes it useful in designing of drug delivery systems (DDS). Physical exercise has consistently been one of the most effective methods for enhancing the body's capabilities. It increases readiness to withstand harmful environmental factors. It offers numerous benefits, including improvements in serum lipid profiles, blood pressure, and inflammatory markers. Additionally, it reduces the risk of stroke, acute coronary syndrome, and overall cardiovascular mortality. HIIT is recognized as a time-efficient strategy for enhancing metabolic health. Studies indicate that HIIT effectively enhances endothelium-dependent vasodilation in individuals with metabolic

syndrome or cardiovascular disorders. Recent research has further demonstrated that HIIT significantly improves oxygen uptake efficiency by enhancing hemodynamic and hem-rheological responses to exercise. Moreover, it suppresses oxidative stress and inflammation associated with cardiac dysfunction in sedentary individuals and patients with heart failure. Circulating endothelial progenitor cells (EPCs) play a crucial role in maintaining endothelial function and organ perfusion through mechanisms that include endothelial repair and neovascularization.⁷ Moreover, HIIT improves circulating levels of TNF- α , leptin, and adiponectin. This suggests that HIIT could be an effective and efficient intervention for managing low-grade inflammation in individuals with metabolic disorders.⁸

Recently, there has been growing concern about extended use of antibiotics uses to chronic wounds. The escalation of bacterial resistance to antibiotics is extensively documented in scientific literature, and it has been widely covered in the media. Finding suitable alternatives to antibiotics that can significantly accelerate the wound healing process., particularly in chronic wounds and burns, is highly crucial. Furthermore, studies consistently indicate that physical exercise enhances the body's readiness and ability to manage pathological condition. Therefore, in the recent study, we aimed to investigate the effects of high-intensity interval training alongside the effects of chitosan hydrogel containing burdock root extract on the wound healing process and its various related factors.

Methods

Experimental Animals

Seventy-two male Wistar rats, aged 2 to 3 months and weighing approximately 240 to 300 grams, were procured from the Laboratory Animal Breeding and Holding Center of the Faculty of Medicine at Mashhad University of Medical Sciences. Immediately upon transfer to the neuroscience department's animal holding facility, they were placed in suitable holding boxes. To reduce relocation stress and acclimatize them to the new environmental conditions, they were kept for 7 days in a room with room temperature, proper ventilation, and a natural light cycle, and with free access to water and food (standard laboratory animal pellets, produced by Javaneh Khorasan Company, Mashhad, Iran).

The rats were randomly divided into four groups of eighteen. The first group (H1) underwent high-intensity interval training for three weeks. The second group (H2) not only underwent the three-week training but also received a topical application of prepared hydrogel twice daily at 12-hours intervals. The third group (H3) consisted of rats that only received the hydrogel treatment throughout the experiment. The fourth group (H4) was the control group, which received neither training nor hydrogel.

Hydrogel

The extraction of burdock root was performed using a 70% hydro alcoholic solution (70% ethanol) through the percolation method.⁽⁹⁾ The extract was standardized using the Folin-Ciocalteu method, ⁽¹⁰⁾ based on total polyphenols, expressed as Gallic acid equivalent, with total polyphenol content of 36.6%.

Two grams of polyvinyl alcohol (PVA; M. W.: 85,000 g/Mol) and one gram of polyethylene glycol (PEG; M.W.: 10000 g/Mol) were dissolved in 50 ml of distilled water over the course of 3 hours. One gram of Chitosan powder (medium molecular weight (190,000–310,000 g/Mol) was dissolved in an acetic acid solution (pH = 4) at 25 °C. The two prepared solutions were mixed together. After thorough mixing, Freeze-dried powder of hydro-alcoholic extract of *Arcticum lappa* root was added to the final solution at 10 % concentration.

All extraction processes and hydrogel preparation were conducted under the supervision of specialists at the Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.

High Intensity Interval Training Exercise (HIIT)

The HIIT exercise regimen consisted of three sessions per week for three weeks, involving treadmill running based on the general principles of High-Intensity Interval Training (HIIT) by Emily Robinson.⁽¹¹⁾ Each session included a 5-minute warm-up and 5-minute cool-down phase 40% of maximum running speed. The main exercise phase consisted of 5 to 7 intervals of 1-minute high-intensity running at 80 to 90% of maximum speed, interspersed with 1-minute intervals of active rest at 55% of maximum speed. All procedures were performed on a treadmill with a zero incline. The detailed HIIT program includes as follows:

Week 1: five intervals of 1-minute high-intensity running at 80% of maximum speed followed by 5 intervals of 1-minute active rest at 55% of maximum running speed.

Week 2: six intervals of 1-minute high-intensity running at 85% of maximum speed followed by 6 intervals of 1-minute active rest at 55% of maximum running speed.

Week 3: seven intervals of 1-minute high-intensity running at 90% of maximum speed followed by 7 intervals of 1-minute active rest at 55% of maximum running speed.

The total exercise duration, including warm-up and cool-down times, was 20 minutes in the first week, 22 minutes in the second week, and 24 minutes in the third week.

To assess maximum exercise capacity and intensity, a maximum running test was conducted in the first week prior to starting the exercise protocol and at the end of the three-week program.

Wound Induction

Anesthesia was induced prior to burn wound induction by administering a combination of medications via intramuscular injection into the posterior calf muscles. The pre-anesthetic included 2.6% ketamine (10%) at a dose of 7.6 mg per kilogram body weight and 1.2% xylazine (2%) at a dose of 5 mg per kilogram body weight. The hair, both sides of spine from below the neck to the middle of the lower back, was shaved. subsequently, anesthesia was induced by 4% isoflurane (Terrell) with oxygen at a flow rate of 6.3 liters per minute, while the maintenance was achieved with 1.5% isoflurane at the same flow rate.

Once anesthesia was confirmed, third-degree burn wounds (affecting epidermis and all layers of the dermis and hypodermis) were induced. The rats were placed dorsoventrally on a flat surface. A copper rod with a circular cross-section and a diameter of 1 centimeter was heated thoroughly in boiling water (100 degrees Celsius for 5 minutes). The heated rod was applied to two points on both sides of rats's backs without pressure for 20 seconds to induce the burns.^{12, 13}

Wound Management

All mice in each group were assessed for general health status and the presence of wound infections. Additionally, their hydration levels and food consumption were monitored for 72 hours after the wound were induced. During the experimental trial, mice in groups H2 and H3 received topical hydrogel application twice daily (every 12 hours), under hygienic conditions.

Macroscopic Wound Assessment

On days 7, 14, and 21, photographs of the wounds of all the remaining mice in each group were taken using a camera. The wound area (in cm²) was measured using Image j software. Subsequently, the percentage of wound contraction on these days was calculated using the following formula:¹²

$$\text{Percentage of wound contraction} = \frac{\text{Area of the original wound} - \text{Wound area on the nth day}}{\text{Area of the original wound}} \times 100$$

Preparation of Hematoxylin and Eosin (H&E) Stained Slides

Skin biopsies for histopathological examination were performed on days 7, 14, and 21 post-burn. Six mice were randomly selected from each group on each of these days and euthanized using sodium pentobarbital. From each euthanized mouse, a 5 mm-thick section of the wound was excised for histopathological examination, placed in 10% formalin buffer, and sent to the pathology laboratory. The samples were prepared in the pathology laboratory using paraffin embedding method, sectioned into 5-micron slides using a microtome, and stained with hematoxylin and eosin according to standard protocol.

Histopathological Examination

The stained slides were analyzed by a pathologist using a light microscope (blind method). Five microscopic slides per sample were examined, and each group was assigned a modified histologic scoring system described by Abramov.¹⁴ The evaluated criteria included angiogenesis, re-epithelization, presence of inflammatory cells, skin collagen status, and fibroplasia.

Data Analysis

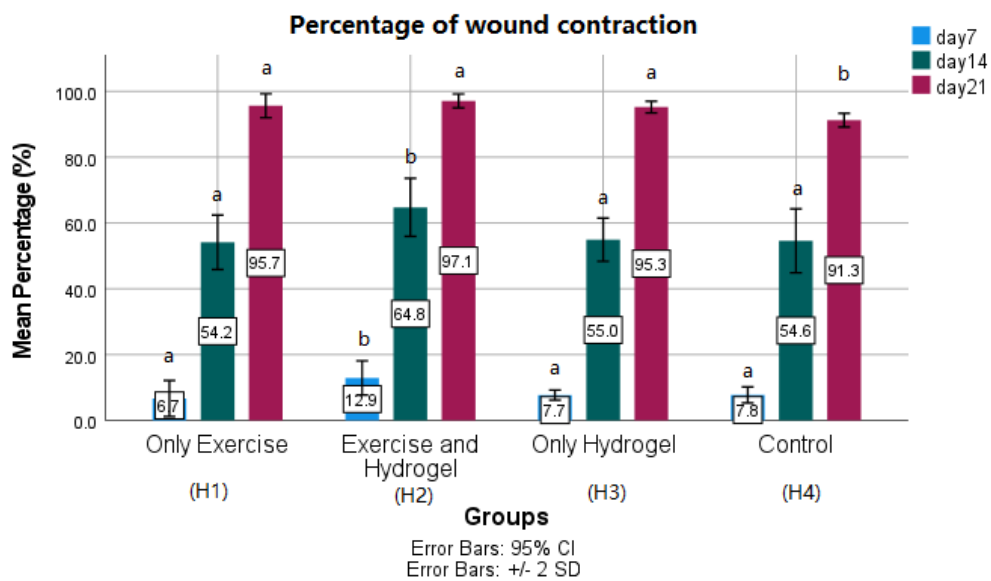
The results were statistically analyzed using SPSS software (IBM SPSS Statistics, V.27, Armonk, New York). A one-way ANOVA test was performed, and the data were expressed as a mean \pm standard deviation. In all evaluations, a p-value of less than 0.05 ($P < 0.05$) was considered statistically significant.

Results

Wound Contraction

On days 7, 14, and 21, the percentage of wound contraction \pm standard deviation was calculated in each group based on measurements obtained from the software and using the specified formula (Table 1). Table 2 presents images of the wound site in each group in each of these days. Graph 1 illustrates the percentage of wound contraction in each group along with their statistically differences. According to the Table 1 and Graph 1, on days 7 and 14, group H2 (chitosan hydrogel

with extract of burdock root and HIIT exercise) showed significant differences in wound contraction compared to the other groups, with values of 12.93 ± 2.60 and 64.76 ± 4.41 , respectively. On day 21, although groups H1, H2, and H3 did not show significant differences statistically among themselves, all three groups exhibited statistically significant differences in wound contraction percentage compared to the control group.



Graph 1: Percentage of wound contraction with statistically differences. a showed no significant differences between groups ($P > 0.05$). b showed significant differences between groups ($P < 0.05$). Statistical analysis by using IBM SPSS V27.0, ANOVA showed significant differences between groups during the study ($P < 0.001$).

Table 1: Macroscopic images of wounds on each day 7,14 and 21

Days/groups	H1	H2	H3	H4
Day 7				
Day 14				
Day 21				

Pathological Assessment

Based on the pathology results, each wound healing factors (re-epithelialization, inflammatory cells, fibroplasia, collagen deposition, and angiogenesis) was individually compared and scored across groups over days 7, 14, and 21, according to the described method. Regarding re-epithelialization, on day 7, groups H2 (chitosan hydrogel with extract of burdock and HIIT exercise) and H3 (hydrogel only) demonstrated moderate re-epithelialization (Figures 2-b and 3-a, respectively), whereas group H1 (exercise only) and the control group showed similar results with mild re-epithelialization (Figures 1-a and 4-a, respectively). On day 14, H2 and H3 again exhibited more extensive re-

epithelialization (severe re-epithelialization; Figures 2-d and 3-d, respectively) compared to the group H1 and the control group, which both showed moderate re-epithelialization (Figures 1-d and 4-c, respectively). By day 21, all three groups (H1, H2, and H3) displayed more advanced re-epithelialization than the control group (all three groups showed severe re-epithelialization, while the control group showed moderate re-epithelialization; Figures 1-f, 2-f and 3-f for the first groups, respectively and Figure 4-e for the control group).

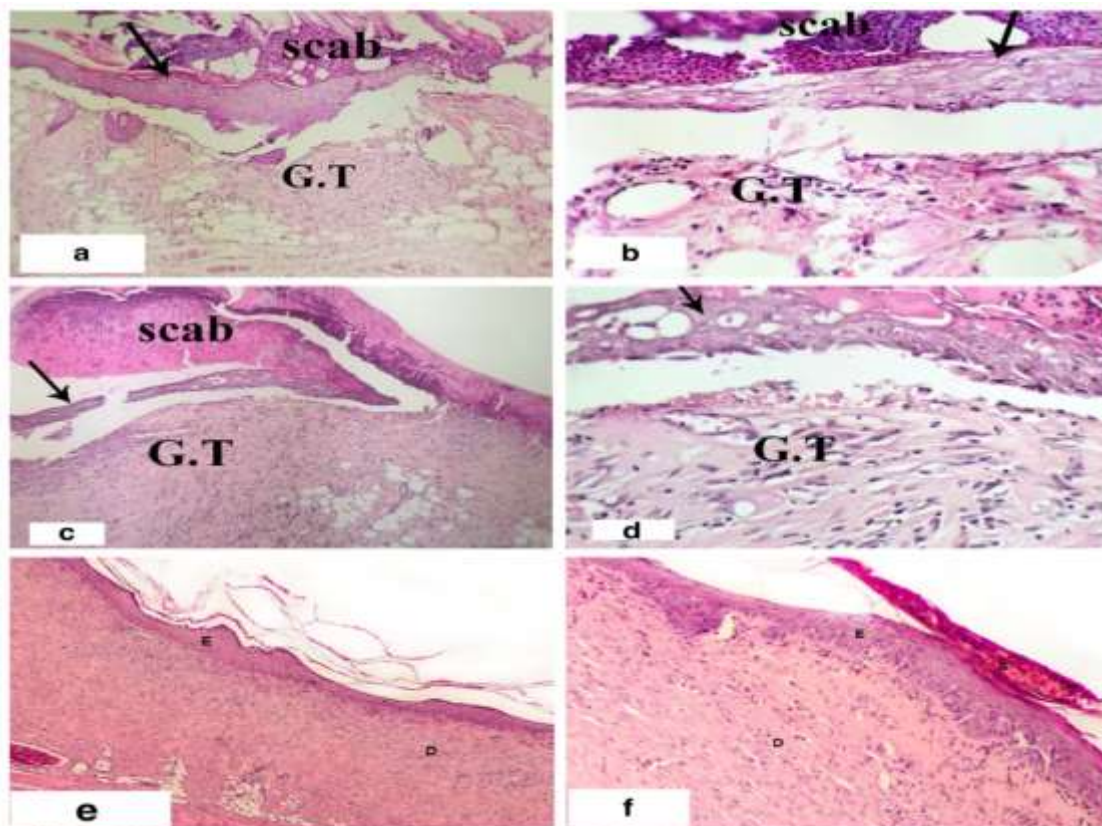


Figure 1: Histopathological examination with light microscopy of group H1. a,b: H1 on day 7th showed a scab formed of necrotic debris and degenerated polymorphonuclear cell (PMNLs); mild re-epithelialization and also granulation tissue consisting of moderate proliferation of fibroblasts, mild presence of inflammatory cells, moderate presence of collagen, moderate newly formed blood vessels under the forming epithelium and residual scab were observed. (a: H & E, $\times 100$) (b: H & E, $\times 400$) (arrow: re-epithelialization) (GT: granulation tissue); c,d: H1 on day 14th showed a scab formed of necrotic debris and degenerated polymorphonuclear cell (PMNLs); Moderate re-epithelialization and also granulation tissue consisting of moderate proliferation of fibroblasts, mild presence of inflammatory cells, severe presence of collagen, moderate newly formed blood vessels under the forming epithelium and residual scab were observed. (c: H & E, $\times 100$) (d: H & E, $\times 400$) (arrow: re-epithelialization) (GT: granulation tissue); e,f: H1 on day 21st indicated mild formed scab of necrotic debris; severe and almost complete re-epithelialization and also dermal layer consisting of moderate proliferation of fibroblasts, mild presence of inflammatory cells (mostly macrophage), moderate presence of collagen, moderate newly formed blood vessels under the formed epithelium were observed. (e: H&E, $\times 40$) (f: H&E, $\times 200$) (E: Epithelial, D: Dermal layer, S: Scab).

In terms of the time required for complete epithelialization to cover the wound surface, groups H2 and H3 nearly achieved complete re-epithelialization by day 7 (Figures 2-b and 3-a, respectively) and reached full maturity and thickness by day 14 (Figures 2-c and 3-c,

respectively). However, group H1 reached this milestone by day 21 (Figure 1-e). In the control group, despite epithelial formation, complete re-epithelialization was not achieved even by day 21 (Figure 4-e).

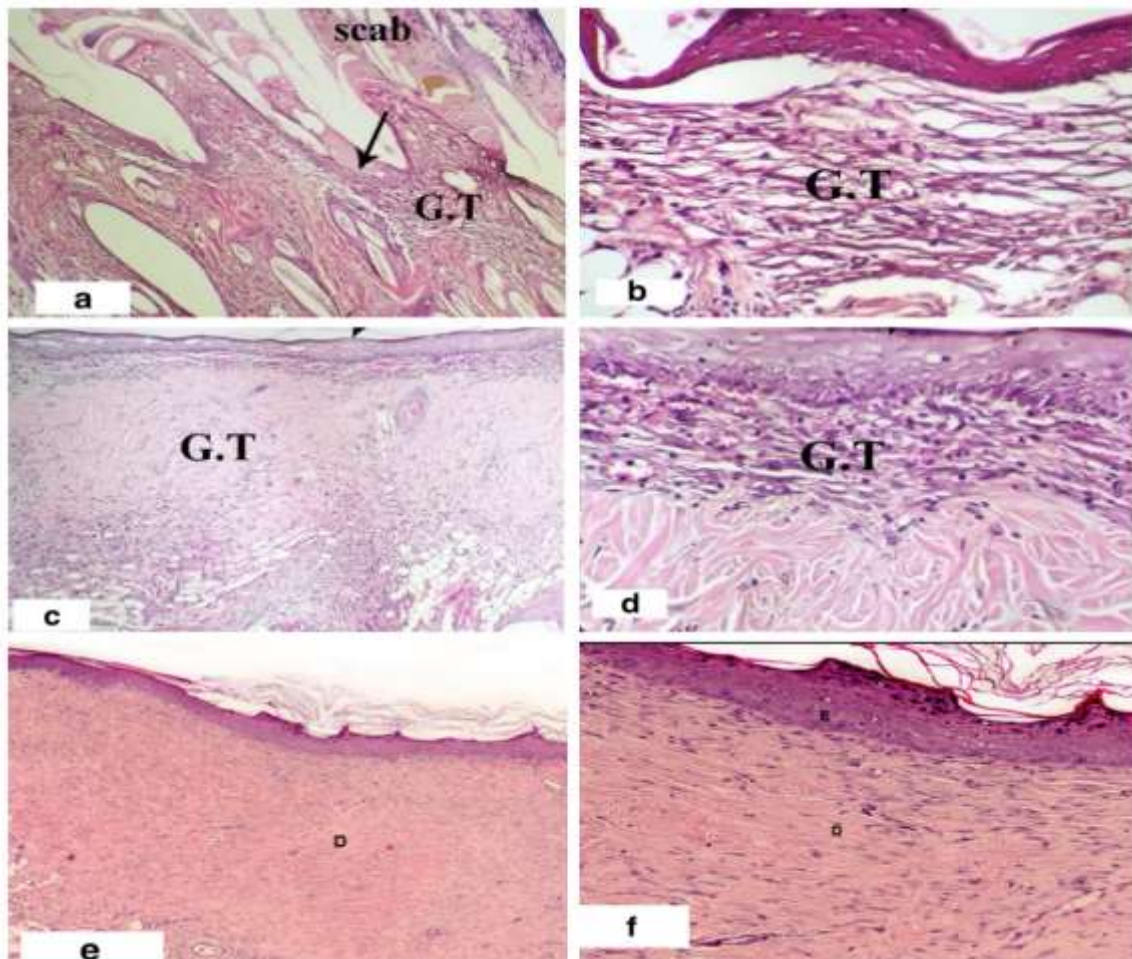


Figure 2: Histopathological examination with light microscopy of group H2. a,b: H2 on day 7th indicated moderate but complete re-epithelialization showing that the newly formed epithelial tissue covers 100% of the wound area with loss and necrosis of the underlying dermal layer, replaced with newly formed granulation tissue with mild presence of inflammatory cells, moderate proliferation of fibroblasts, moderate presence of collagen and moderate newly formed blood vessels. (a: H & E, $\times 100$) (b: H & E, $\times 400$) (arrow: Epithelialization) (GT: granulation tissue); c,d: H2 on day 14th showed the newly formed epithelial tissue covers 100% of the wound area with loss and necrosis of the underlying dermal layer, replaced with newly formed granulation tissue with moderate presence of inflammatory cells, severe proliferation of fibroblasts, severe presence of collagen and severe newly formed blood vessels. (c: H & E, $\times 100$) (d: H & E, $\times 400$) (arrow: Epithelialization) (GT: granulation tissue); e,f: H2 on day 21st showed that the newly formed epithelial tissue covers 100% of the wound area with underlying dermal layer, with mild presence of inflammatory cells, severe proliferation of fibroblasts, severe presence of collagen and severe newly formed blood vessels. (e: H&E, $\times 40$) (f: H&E, $\times 200$) (E: Epithelial, D: Dermal layer).

Concerning presence of inflammatory cells, on days 7 and 21, all three groups (H1, H2 and H3) exhibited a mild presence of inflammatory cells, primary macrophage (Figures 1-b, 1-f and 2-b, 2-f and 3-b, 3-f, respectively), In contrast, the control group showed a severe presence of inflammatory

cells on day 7, and a moderate presence by day 21 (Figures 4-b and 4-f, respectively). Inflammatory cells in H2 increased somewhat on day 14 (Figure 2-d) but remained mild on day 21 (Figure 2-f). The level of inflammatory cells in the control group decreased over days 14 and 21.

Regarding fibroplasia, on day 7, mild proliferation of fibroblasts was observed in the control group, and H3 (Figures 4-b and 3-b, respectively), while H1 and H2 showed moderate fibroplasia (Figures 1-b and 2-b, respectively). On day 14, severe fibroplasia was present in groups H2 and H3 (Figures 2-d and 3-d, respectively), whereas H1 and

the control group showed moderate levels (Figures 1-d and 4-d, respectively). On day 21, groups H1, H3, and the control group showed moderate fibroplasia (Figures 1-f, 3-f and 4-f, respectively), with only H2 displaying severe fibroplasia (Figure 2-f).

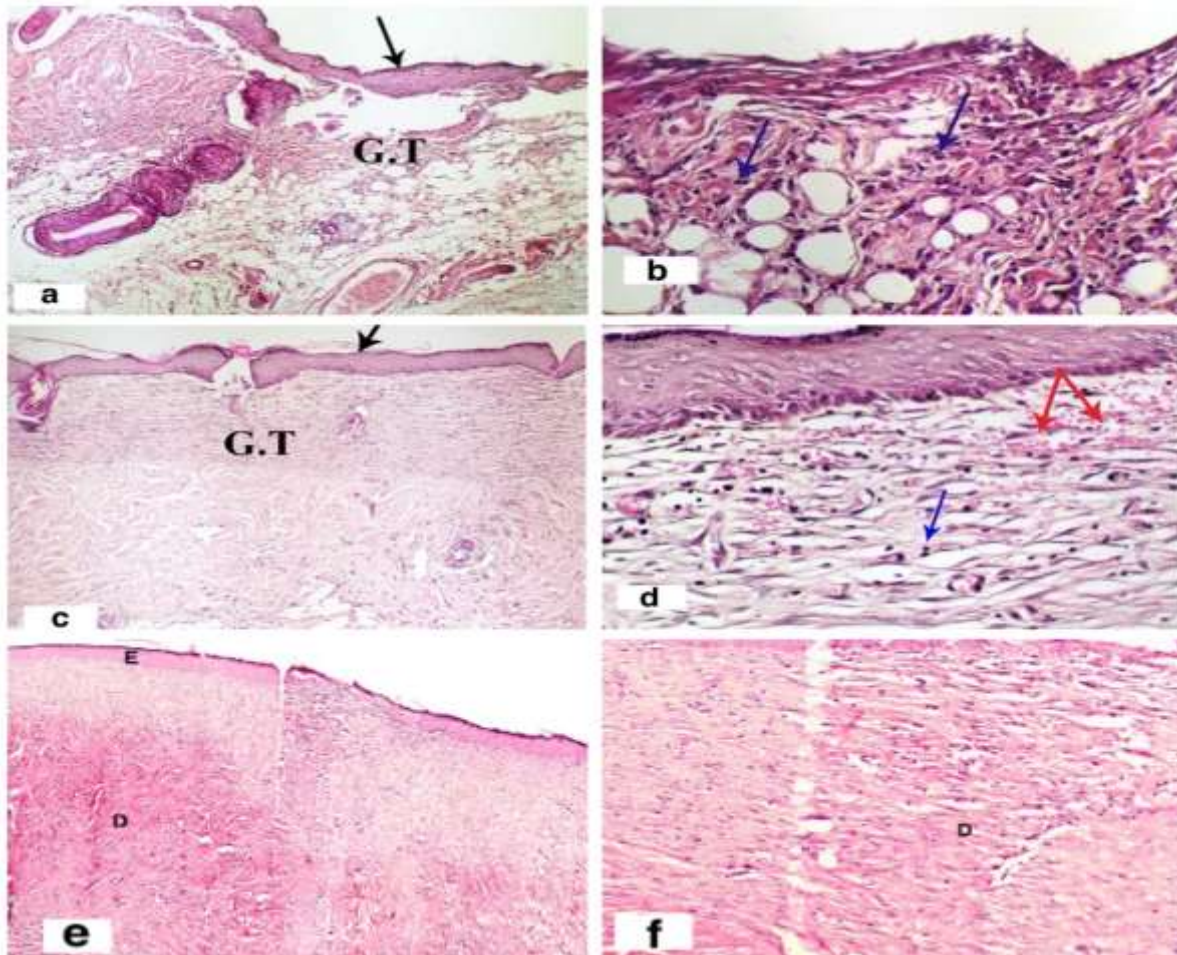


Figure 3: Histopathological examination with light microscopy of group H3. a,b: H3 on day 7th showed that moderate but complete re-epithelialization showing that the newly formed epithelial tissue covers 100% of the wound area with loss and necrosis of the underlying dermal layer, replaced with newly formed granulation tissue with mild presence of inflammatory cells (mostly macrophage), mild proliferation of fibroblasts, moderate presence of collagen and mild newly formed blood vessels. (a: Hematoxylin and Eosin, $\times 100$), (b: Hematoxylin and Eosin, $\times 400$), (black arrow: Epithelialization), (GT: granulation tissue), (blue arrow: inflammatory cells); c,d: H3 on day 14th indicated complete re-epithelialization showing that the newly formed epithelial tissue covers 100% of the wound area with loss and necrosis of the underlying dermal layer, replaced with newly formed granulation tissue with mild presence of inflammatory cells (mostly macrophages), severe proliferation of fibroblasts, severe presence of collagen and severe newly formed blood vessels. (c: H & E, $\times 100$, black arrow: Epithelialization), (d: H & E, $\times 400$, black arrow: inflammatory cells), (GT: granulation tissue), (red arrow: newly formed blood vessels), (blue arrow: inflammatory cells); e,f: H3 on day 21st showed complete re-epithelialization showing that the newly formed epithelial tissue covers almost 100% of the wound area with the underlying dermal layer with mild presence of inflammatory cells, moderate proliferation of fibroblasts, severe presence of collagen and severe newly formed blood vessels. (e: H&E, $\times 40$), (f: H&E, $\times 200$), (E: Epithelial, D: Dermal layer).

On the subject of collagen deposition, on day 7, all four groups showed moderate levels of collagen deposition (Figures 1-b, 2-b, 3-b and 4-b,

respectively). On day 14, H1, H2, and H3 exhibited severe collagen deposition (severe; Figures 1-d, 2-d and 3-d, respectively) while the control group

remained at a moderate level (Figure 4-d). However, by day 21, collagen deposition remained severe in groups H2 and H3 (Figures 2-f and 3-f, respectively), while it was moderate in group H1 and the control group (Figures 1-f and 4-f, respectively).

Finally, as regards angiogenesis, H3 and the control group showed mild levels of angiogenesis on days 7th (Figures 3-b and 4-b, respectively). While the control group maintained this mild level through

day 14 (Figure 4-d), it increased to moderate levels by day 21st (Figure 4-f). In contrast, groups H2 and H3 exhibited severe angiogenesis on days 14th (Figures 2-d and 3-d, respectively) and 21st (Figures 2-f and 3-f, respectively). On day 7, H2 and H3 had moderate and mild levels of angiogenesis, individually (Figures 2-b and 3-b, respectively). Group H1 consistently showed moderate levels of angiogenesis throughout the experimental period (Figures 1-b, 1-d and 1-f, respectively).

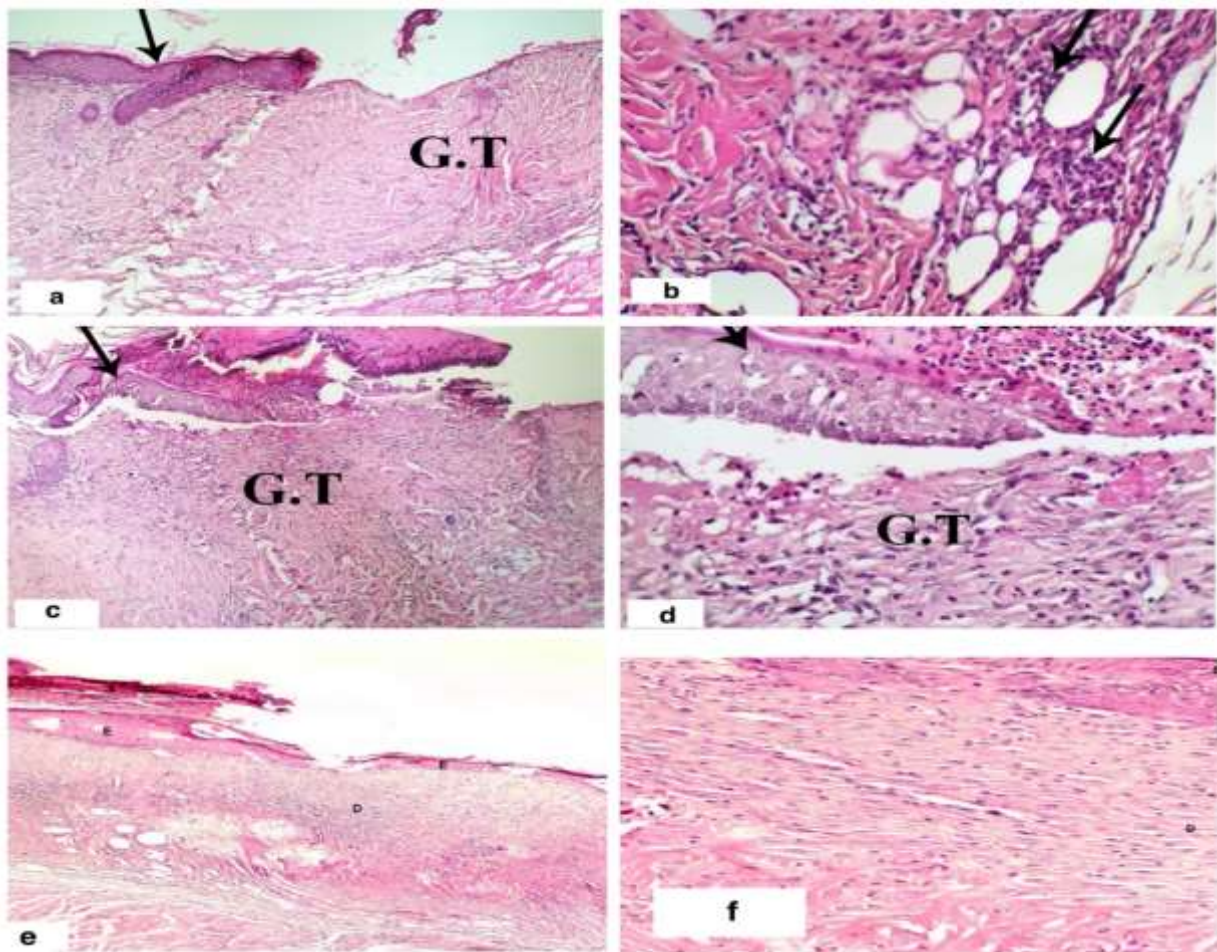


Figure 4: Histopathological examination with light microscopy of group H4. a,b: H4 on day 7th showed a scab formed of necrotic debris and degenerated polymorphonuclear cell (PMNLs); mild re-epithelialization and also granulation tissue consisting of mild proliferation of fibroblasts, severe presence of inflammatory cells, moderate presence of collagen, mild newly formed blood vessels under the forming epithelium and residual scab were observed. (a: H & E, $\times 100$, arrow: re-epithelialization), (b: H & E, $\times 400$, arrows: inflammatory cells), (GT: granulation tissue); c,d: H4 on day 14th showed a scab formed of necrotic debris and degenerated polymorphonuclear cell (PMNLs); moderate re-epithelialization and also granulation tissue consisting of moderate proliferation of fibroblasts, mild presence of inflammatory cells, moderate presence of collagen, mild newly formed blood vessels under the forming epithelium and residual scab were observed. (c: Hematoxylin and Eosin, $\times 100$, arrow: re-epithelialization), (d: Hematoxylin and Eosin, $\times 400$, arrows: inflammatory cells), (GT: granulation tissue); e,f: H4 on day 21st indicated a scab formed of necrotic debris and degenerated polymorphonuclear cell (PMNLs); moderate and uncompleted re-epithelialization and also dermal layer consisting of moderate proliferation of fibroblasts, moderate presence of inflammatory cells, moderate presence of collagen, moderate newly formed blood vessels under the forming epithelium and residual scab were observed. (e: H&E, $\times 40$), (f: H&E, $\times 200$), (E: Epithelial, D: Dermal layer, S: Scab).

Discussion

The skin is the largest organ of the body, and serves as the first barrier against various environmental traumas, including physical, chemical, and biological threats. It also plays crucial roles in maintaining body temperature and hydration. Consequently, any damage to the skin poses significant risks from external environmental hazards. Burn wounds are among the most critical types of skin traumas, which can even threaten lives, and complicate the healing process. Due to their chronic nature, these wounds often require extensive and costly treatment. Generally, traumatic wounds are classified into two categories based on their healing duration: acute and chronic. Most acute wounds heal completely within 4 to 6 weeks. However, wounds that take longer than 6 weeks to heal are termed as a chronic wound. Researchers have continuously sought ways to reduce healing time, even for acute wounds, while also emphasizing the importance of the healed wound's quality in comparison to natural skin, both functionally and aesthetically. Therefore, various studies have examined different factors of wound healing, such as pathological characteristics. Recent studies have shown that using chitosan hydrogel combined with burdock root extract can independently enhance the duration and quality of wound healing by affecting various stages of the healing process. Additionally, different types of exercise contribute to overall health and body fitness. Our results indicated that, compared to the control group, the quality and duration of wound healing, as well as rehabilitation time, were significantly improved in the group subjected to high-intensity interval training (HIIT). Moreover, the combined use of hydrogel and exercises demonstrated a synergistic effect, yielding optimal performance and faster rehabilitation in the group where both methods were employed.

Wound healing generally comprises four stages. The first stage is hemostasis or coagulation, during which the damaged blood vessels are blocked by blood clots facilitated by platelets to prevent further bleeding and the entry of pathogens. These platelets release various cytokines that attract inflammatory cells to the wound site. The next stage is the inflammatory phase, characterized by the secretion of cytokines, chemokines, and various inflammatory mediators by the damaged cells and platelets in the wound environment. This secretion attracts various inflammatory cells, including

leukocytes, to the wound site. Neutrophils, one of the most important leukocytes, eliminate pathogens and clear cellular debris from the wound. After fulfilling the wound, neutrophils either return to the main bloodstream or undergo apoptosis. Monocytes migrate to the wound site through diapedesis and transform into macrophages. Initially, these macrophages maintain an inflammatory environment by secreting various mediators. In the latter stages, macrophages secrete anti-inflammatory cytokines to reduce inflammation, paving a way for angiogenesis, re-epithelialization, and collagen synthesis. The activity of macrophages is critical in wound healing, and a shorter inflammatory phase. Thus, the fewer environmental contaminants present (meaning fewer neutrophils needed for cleaning and for shorter durations), or the quicker the wound progresses through the inflammatory phase, the faster it can enter the proliferation phase. The third stage of wound healing is the proliferation phase, involving intense activity of keratinocytes, fibroblasts, macrophages, and endothelial cells, which leads to wound closure, collagen synthesis, and angiogenesis. The quality of wound healing is determined at this stage. For instance, the more granulation tissue produced by fibroblasts, the more angiogenesis and collagen are generated, resulting in faster and higher-quality wound healing. Additionally, fibroblasts accumulate at the wound edges and contract to bring them closer together, a process known as wound contraction. The final stage is the remodeling phase, where the structure and shape of fibroblasts and collagen change, eventually forming scar tissue.^{1, 15, 16} The recent study, based on macroscopic and microscopic examinations, primarily focused on two crucial phases of wound healing: the inflammatory and proliferation phases. The detailed impact of chitosan hydrogel containing burdock extract and high-intensity interval training (HIIT) on these phases is thoroughly explained in the subsequent paragraph.

As previously mentioned, the inflammatory phase is one of the initial stages of wound healing, marked by various inflammatory cells, including neutrophils and macrophages. Naturally, the more environmental contamination there is, the longer this phase—particularly the presence of neutrophils, the secretion of inflammatory cytokines, and the phagocytic action of macrophages—will last, delaying the wound healing

process. In other words, in wound management, the cleaner and less infected the wound environment is kept, the shorter the inflammatory process will be. In fact, one of the most important reasons for a wound becoming chronic is the prolonged inflammatory phase. A crucial issue in traumatic burn wounds is the loss of the skin's natural flora, increasing the possibility of severe infection and inflammation.¹⁷ The use of dressings and bandages with antimicrobial and anti-inflammatory properties significantly aids in accelerating the wound healing process. Recent studies have shown that hydrogels containing chitosan exhibit anti-inflammatory and antibacterial properties, in which help to reduce infection and inflammation at the wound site.^{6, 18} Additionally, due to the various compounds found in burdock plant extracts, particularly the root, its anti-inflammatory, antimicrobial, and antioxidant effects have been noted in the treatment of skin diseases. For example, the extract of this plant inhibits the production of nitric oxide synthase (iNOS) and nitric oxide (NO), subsequently reducing the synthesis of pro-inflammatory cytokines via the nuclear factor-kappa B (NF- κ B) pathway, activating antioxidant enzymes, and scavenging free radicals, ultimately significantly reducing inflammation. The extract of burdock has been shown to exhibit anti-inflammatory responses by inhibiting the degranulation and release of cysteinyl leukotrienes (Cys-LTs) by peripheral blood mononuclear cells (PBMCs). Cys-LTs are synthesized inflammatory mediators such as histamine and prostaglandins. The blockade of Cys-LT is regarded as the inhibition of the inflammatory response. Another component of burdock extract is polyphenols, which have been attributed a range of biological activities, including anti-inflammatory, immunomodulatory, antioxidant, and anticancer properties. Studies have reported that polyphenols target receptors on immune cells such as mast cells, neutrophils, monocytes/macrophages, and T cells. The immunomodulatory properties of these substances can be prominent in wound management, improving neutrophil function in combating environmental pathogens that infect wounds, and influencing macrophages in producing anti-inflammatory cytokines that play a role in angiogenesis, re-epithelialization, and collagen production. Additionally, since oxidative stress negatively affects fibroblast activity, the antioxidant properties of this plant extract can aid in faster wound

healing.^{2, 3, 17, 19, 20} Another significant aspect is the impact of exercise on reducing inflammation during the wound healing process. Studies have shown that exercise (especially HIIT) can increase the levels of endothelial micro particles (EMP). EMPs are complex vesicular structures, which shed from activated or apoptotic endothelial cells and are considered significant in coagulation, inflammation, endothelial function, and angiogenesis.^{7, 21} EMPs are one of the effective factors resulting from exercise in reducing inflammation levels in the body. All the mentioned properties of burdock plant extract, hydrogel, and the effects of exercise lead us to expect a reduction in inflammation during the wound healing process. According to our study results, the presence of inflammatory cells (especially neutrophils) in the groups subjected to high-intensity interval training (H1), and those treated with chitosan hydrogel containing burdock root extract (H3) was significantly lower than in the control group. Furthermore, the synergistic effect of simultaneously using this hydrogel and the HIIT program in group H2 resulted in a notable reduction in inflammation, particularly in the first week of wound healing, was notable. Although it is difficult to declare a precise difference between these three groups, it can be concluded that all three groups showed better performance compared to the control group during the study period (especially in the first week of wound healing due to the occurrence of the inflammatory phase). Ultimately, the results demonstrated the anti-inflammatory and antimicrobial effects of the burdock root extract.

One of the most crucial stages of wound healing, during which the body works to repair and create new structures, is the proliferation phase. Present study aimed to investigate the effects of chitosan hydrogel containing burdock root extract, as along with HIIT, on this phase from both macroscopic (percentage of wound contraction) and microscopic perspectives. Therefore, we examined the pathological characteristics of this phase (fibroplasia, angiogenesis, collagen deposition, and re-epithelialization) in detail below.

In the proliferation phase, fibroblasts contribute to wound healing by forming granulation tissue at the wound site, which promotes angiogenesis, collagen production, skin regeneration, and ultimately complete wound closure. Conversely, the accumulation of fibroblasts at the wound edge contributed to wound

contraction and closure, reducing the size of the initial wound. Therefore, evaluating the presence and proliferation of fibroblasts and the extent of angiogenesis in the wound environment is crucial to assess the impact of the mentioned hydrogel and HIIT. According to the results of present study, during the first week, the proliferation of fibroblasts and angiogenesis in the group H3 (hydrogel) was comparable to the control group (mild proliferation), whereas these levels were higher in the group H1 (high-intensity interval training) and group H2 (hydrogel combined with exercise) compared to the other groups (moderate proliferation). The possible reason for the higher fibroplasia and angiogenesis in these two groups could be attributed to the higher level of lactic acid in the wound environment. Generally, the wound environment is anaerobic, and in the early stages of wound healing, anaerobic metabolism and lactate dehydrogenase (LAD) play a significant role in providing energy and stimulating the proliferation of fibroblasts, macrophages, and angiogenesis. Studies have shown that lactic acid in the wound side enhances fibroplasia and angiogenesis.^{22, 23} Furthermore, high-intensity exercise can increase anaerobic metabolism in cells, leading to the production of lactic acid in cells and interstitial fluid. Since the mice in the group H1 had recently undergone high-intensity interval training in the first week, it is possible that endothelial cells and interstitial fluid in this group had higher levels of lactic acid. Therefore, the higher proliferation of fibroblasts in the group H1 during the first week may be attributed to this factor. However, proving this requires further research, including measuring the level of lactic acid in the wound environment in a similar study. Furthermore, high-intensity exercise can increase the level of vascular endothelial growth factor (VEGF) in skin injuries, which can further stimulate angiogenesis.^{7, 21, 23} Considering that the proliferation of fibroblasts and angiogenesis in the group H3 did not differ significantly from the control group in the first week, the higher level of fibroblast proliferation and angiogenesis in the group H2 could be attributed to the effects of HIIT, lactic acid, and VEGF. Moreover, although it has been suggested that specific doses of Burdock extract have negative effects on fibroblasts,²⁴ other studies confirm the positive effects of this plant extract on fibroblasts.⁴ For example, it has been established that the polyphenols present in Burdock extract prevent the deleterious

effects of oxidative stress and free radicals on fibroblasts due to their antioxidant properties (fibroblasts are among the primary targets of free radicals during oxidative stress). Furthermore, the role of chitosan in promoting the migration of fibroblasts has been mentioned in this study.¹⁷ Additionally, the positive effect of chitosan present in hydrogel on fibroblasts and angiogenesis cannot be overlooked.^{5, 6} In a study, chitosan-alginate hydrogel provided a favorable environment for the growth and proliferation of L929 fibroblast cells of mice, leading to increased proliferation.²⁵ Another study demonstrated that the use of hydrogel containing chitosan had a positive impact on angiogenesis in skin wounds.⁵ Therefore, it might be attributed to the positive effects of polyphenols present in Burdock plant extract and chitosan in the hydrogel that severe proliferation of fibroblasts and increased angiogenesis in the second week were observed in groups H2 and H3 (Figure 3-d) compared to other groups in the recent study. As mentioned earlier, wound-edge fibroblasts cause wound contraction and size reduction. According to recent study results, there was a statistically significant difference ($P < 0.05$) in the percentage of wound contraction during the first week between the H2 group and other groups, while fibroblast proliferation rate was approximately similar between groups H1 and H2 during the first week. An important point in the second week is that fibroblast proliferation rates were reported as severe in both groups H2 and H3; however, there was still a statistically significant difference in wound contraction percentage in the second week in the H2 group compared to other groups. Given that high-intensity exercise enhances wound healing and wound contraction,²⁶ these statistically significant differences may indicate the synergistic effect of using this hydrogel alongside high-intensity interval training. Furthermore, despite severe proliferation of fibroblasts in the group H2 in the third week, no statistically significant difference in wound contraction was reported among this group and the groups H1 and H3. All three groups, however, showed statistically significant differences compared to the control group, indicating that ultimately, the use of chitosan hydrogel containing Burdock plant extract and high-intensity interval training could lead to greater wound contraction and smaller wound size, as well as potential synergistic effects. In a similar study on excision wounds, the percentage of wound contraction

in a group using chitosan hydrogel without any additional active substances was higher on days 7 and 14 compared to the control group, although no statistically significant difference was observed between them.²⁷

Collagen deposition is another critical factor in wound healing that was examined in the recent study. As previously noted, collagen production by fibroblasts during the proliferative phase is a vital for effective wound repair. Studies have shown that Burdock extract, through various mechanisms such as its antioxidant properties in protecting fibroblasts from oxidative stress, can enhance collagen deposition in wound healing.¹⁷ Another study indicated that Burdock root extract increased collagen production in wounds.²⁸ Additionally, hypoxia and lactic acid production in the body prior to wound induction can stimulate greater collagen production and fibroblast accumulation, with levels peaking around day 14th.^{22, 23} Another study on wounds showed that chitosan in the hydrogel could also stimulate collagen production.⁶ Despite these findings, collagen deposition levels in the first week showed no significant difference among the three experimental groups and the control group (all showing moderate collagen deposition). However, in the subsequent weeks, collagen deposition levels changed noticeably. In the second and third weeks, collagen deposition in groups H1, H2, and H3 was higher than in the control group. This increase might be attributed to the effects of the plant extract, lactic acid, and the higher fibroblast proliferation in these groups.

Skin regeneration and the completion of healing process are of utmost importance, as delayed re-epithelialization increases the likelihood of infection and delays wound recovery. This process is highly influenced by both external factors such as environmental hygiene, wound location, physical pressure applied to the wound, and moisture levels on the wound surface. Additionally, internal factors such as infection and inflammation levels at the wound site, fibroblast presence and proliferation, angiogenesis, and blood supply all interdependently affect the rate of re-epithelialization. Optimal conditions for these factors enable faster skin regeneration and closure. In the recent study, HIIT and the use of a chitosan hydrogel containing Burdock root extract were found to positively influence faster re-epithelialization. These findings align with previous research demonstrating the beneficial effects of chitosan

hydrogel^{5, 6, 27} and exercise^{21, 22, 26} on re-epithelialization. In the present study, skin regeneration in the control group, despite moderate levels of re-epithelialization, was not complete by day 21st. In contrast, skin regeneration in the group H1 was completed by this day. Interestingly, in the groups H2 and H3, despite moderate levels of re-epithelialization, they nearly achieved complete re-epithelialization by day 7th and reached full maturity and thickness by day 14th. Therefore, it can be concluded from the recent study that the used hydrogel had a greater impact on the re-epithelialization process than the exercise regimen.

Conclusion

The present study investigated the effects of chitosan hydrogel containing the root extract of *Arctium lappa* (Burdock) on the traumatic Burn Wound Healing in Rats undergoing HIIT Exercise. The results showed that the combined application of chitosan hydrogel with burdock root extract and high intensity interval training markedly improved wound healing outcomes. This was evidenced by enhanced wound contraction, re-epithelialization, fibroplasia, collagen deposition and angiogenesis, along with a well-modulated inflammatory response. The findings highlight the potential of this combined therapeutic approach in promoting more efficient and effective wound healing.

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Conflict of Interest Disclosures

The authors declare no conflicts of interest.

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Authors' Contributions

All authors contributed equally to accomplishing this study.

Ethical Statement

All applicable international, national, or institutional guidelines for the care and use of animals were

followed. The research protocol obtained ethical approval from Baqiyatallah University of Medical Sciences, designated by the code IR.BMSU.AEC.1401.012.

References

- Flanagan M. The physiology of wound healing. *Journal of wound care*. 2000;9(6):299-300.
- Chan Y-S, Cheng L-N, Wu J-H, Chan E, Kwan Y-W, Lee SM-Y, et al. A review of the pharmacological effects of *Arctium lappa* (burdock). *Inflammopharmacology*. 2011;19(5):245-54.
- Duh PD. Antioxidant activity of burdock (*Arctium lappa* Linne): its scavenging effect on free-radical and active oxygen. *Journal of the American Oil Chemists' Society*. 1998;75(4):455-61.
- Pomari E, Stefanon B, Colitti M. Effect of *Arctium lappa* (burdock) extract on canine dermal fibroblasts. *Veterinary Immunology and Immunopathology*. 2013;156(3-4):159-66.
- Wei L, Tan J, Li L, Wang H, Liu S, Chen J, et al. Chitosan/alginate hydrogel dressing loaded FGF/VE-cadherin to accelerate full-thickness skin regeneration and more normal skin repairs. *International journal of molecular sciences*. 2022;23(3):1249.
- Wang T, Zhu X-K, Xue X-T, Wu D-Y. Hydrogel sheets of chitosan, honey and gelatin as burn wound dressings. *Carbohydrate polymers*. 2012;88(1):75-83.
- Tsai H-H, Lin C-P, Lin Y-H, Hsu C-C, Wang J-S. High-intensity interval training enhances mobilization/functionality of endothelial progenitor cells and depressed shedding of vascular endothelial cells undergoing hypoxia. *European journal of applied physiology*. 2016;116:2375-88.
- Khalafi M, Symonds ME. The impact of high-intensity interval training on inflammatory markers in metabolic disorders: A meta-analysis. *Scandinavian journal of medicine & science in sports*. 2020;30(11):2020-36.
- Singh J. Maceration, percolation and infusion techniques for the extraction of medicinal and aromatic plants. Extraction technologies for medicinal and aromatic plants. 2008;67:32-5.
- Lamuela-Raventós RM. Folin–Ciocalteu method for the measurement of total phenolic content and antioxidant capacity. Measurement of antioxidant activity & capacity: recent trends and applications. 2018:107-15.
- Jung ME, Bourne JE, Beauchamp MR, Robinson E, Little JP. High-intensity interval training as an efficacious alternative to moderate-intensity continuous training for adults with prediabetes. *Journal of diabetes research*. 2015;2015(1):191595.
- Mansoub NH, Gьrdal M, Karadaş E, Kabadayi H, Vatansver S, Ercan G. The role of PRP and adipose tissue-derived keratinocytes on burn wound healing in diabetic rats. *BiolImpacts: BI*. 2018;8(1):5.
- Sharma R, Yeshwante S, Valli Q, Hussein M, Thombare V, McCann SM, et al. Rat Burn Model to Study Full-Thickness Cutaneous Thermal Burn and Infection. *JoVE (Journal of Visualized Experiments)*. 2022(186):e64345.
- Gurgьl T, Olgun A, Torun M, Kargi E. Application of N-butyl cyanoacrylate to split-thickness skin grafts in rats: an experimental study. *Dermatologic Surgery*. 2015;41(9):1024-9.
- Peate I. The inflammatory process and skin healing. *British Journal of Healthcare Assistants*. 2011;5(12):584-7.
- Shedoeva A, Leavesley D, Upton Z, Fan C. Wound healing and the use of medicinal plants. *Evidence-Based Complementary and Alternative Medicine*. 2019;2019(1):2684108.
- Fernandes A, Rodrigues P, Pintado M, Tavaría F. A systematic review of natural products for skin applications: Targeting inflammation, wound healing, and photo-aging. *Phytomedicine*. 2023;115:154824.
- Yuan N, Shao K, Huang S, Chen C. Chitosan, alginate, hyaluronic acid and other novel multifunctional hydrogel dressings for wound healing: A review. *International Journal of Biological Macromolecules*. 2023;240:124321.
- Aitynova A, Ibragimova N, Shalakhmetova T, Gapurkhaeva T, Krasnoshtanov A, Kenesheva S. Antimicrobial effect of extract from root of *Arctium tomentosum* Mill.(woolly burdock) against several reference strains. *International Journal of Biology and Chemistry*. 2022;15(2):10-7.
- Pirvu L, Nicorescu I, Hlevca C, Albu B, Nicorescu V. Burdock (*Arctium lappa*) leaf extracts increase the in vitro antimicrobial efficacy of common antibiotics on gram-positive and gram-negative bacteria. *Open Chemistry*. 2017;15(1):92-102.
- Wahl P, Jansen F, Achtzehn S, Schmitz T, Bloch W, Mester J, et al. Effects of high intensity training and high volume training on endothelial microparticles and angiogenic growth factors. *PLoS one*. 2014;9(4):e96024.
- Hunt TK, Conolly WB, Aronson SB, Goldstein P. Anaerobic metabolism and wound healing: an hypothesis for the initiation and cessation of collagen synthesis in wounds. *The American Journal of Surgery*. 1978;135(3):328-32.
- Trabold O, Wagner S, Wicke C, Scheuenstuhl H, Hussain MZ, Rosen N, et al. Lactate and oxygen constitute a fundamental regulatory mechanism in wound healing. *Wound repair and regeneration*. 2003;11(6):504-9.
- Rieman MT, Neely AN, Boyce ST, Kossenjans WJ, Durkee PJ, Zembrod JM, et al. Amish burn ointment and burdock leaf dressings: assessments of antimicrobial and cytotoxic activities. *Journal of Burn Care & Research*. 2014;35(4):e217-e23.
- Baysal K, Aroguz AZ, Adiguzel Z, Baysal BM. Chitosan/alginate crosslinked hydrogels: Preparation, characterization and application for cell growth purposes. *International journal of biological macromolecules*. 2013;59:342-8.
- Kulprachakarn K, Nantakool S, Rojawat C, Ounjaijean S, Pongtam S, Prasannarong M, et al. Effectiveness of combined conventional treatment with a tailored exercise training program on wound healing in patients with venous leg ulcer: A randomized controlled trial. *Journal of tissue viability*. 2022;31(1):190-6.
- Ehterami A, Salehi M, Farzamfar S, Samadian H, Vaez A, Ghorbani S, et al. Chitosan/alginate hydrogels containing Alpha-tocopherol for wound healing in rat model. *Journal of Drug Delivery Science and Technology*. 2019;51:204-13.
- Ghorat F, Azizkhani M, Naji S, Ranjbary AG, Doostishoa F. Histopathological Evaluation of Burdock (*Arctium lappa*) Root Hydroalcoholic Extract on Wound Healing. *Iranian Red Crescent Medical Journal*. 2017;19(1).