Original Research (XXXX) X(X); X - XX
https://doi.org/10.22103/VCBR.2025.25132.1064

Shahid Bahonar University of Kerman

Veterinary and Comparative Biomedical Research

ORIGINAL ARTICLE

The Effect of Liposome Nanocarrier Containing 2% *Arnebia euchroma* Extract on Infectious Burn Wound Healing in Rats

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Online ISSN: 3060-7663 **https://doi.org/10.22103/VCBR.2025.25132.1064**

Article History

Received: *23 April 2025*
Revised: *07 May 2026*
Accepted: *07 June 2026*
Published: *11 July 2026*

Keywords

Arnebia euchroma
Infectious Wound Healing
Rat
Histopathology
Nano-Liposomes
Hyaluronic acid

Abstract

This study investigated the effect of a hyaluronic acid-based hydrogel containing 2% *Arnebia Euchroma* extract-loaded nanoliposomes on the healing of second-degree burn wounds infected with Pseudomonas aeruginosa in rats. Twenty-four male Wistar albino rats (48 wounds total) were randomly divided into six groups of eight. Second-degree burns were induced on the right and left flanks of each rat using a 1 cm diameter, 100-gram copper rod heated to 100°C. The wounds were then infected with Pseudomonas aeruginosa and covered with a dressing for 48 hours. Treatment commenced on subsequent days. On days 9 and 15, half of the animals from each group were euthanized, and skin samples were collected. Histopathological parameters, including inflammation, granulation tissue formation, collagen content, epidermal regeneration, and angiogenesis, were evaluated. Evaluation of wound healing at days 9 and 15 revealed that the positive control group and the standard treatment group exhibited the greatest improvement compared to other groups. However, the group treated with Hydroalcoholic extract of *Arnebia Euchroma* displayed a different healing pattern. On day 9, this group outperformed other groups in some evaluated parameters, but on day 15, wound healing in this group was slower. These findings suggest that the hyaluronic acid hydrogel containing *Arnebia Euchroma* extract-loaded nanoliposomes has significant potential for accelerating the healing of superficial infected burn wounds.

How to cite this article: Mohammadmahdi Alinaghizadeh, Mohammad Mahdi Molaei, Ehsanollah Sakhaee, Reza Kheirandish , Pourya Mohammadi, Fatemeh Heydari, Reza Molaei, Fateme Mohebbi pourkani The Effect of Liposome Nanocarrier Containing 2% *Arnebia euchroma* Extract on Infectious Burn Wound Healing in Rats. *Veterinary and Comparative Biomedical Research*, XXXX X(X): X – XX. https://doi.org/10.22103/VCBR.2025.25132.1064

Introduction

The skin, which is the largest organ of the body, serves as the first barrier against harm (1). When there is a disruption in the layers of the skin, specifically the dermis and epidermis, it is classified as a wound (2). Damage caused by fire, intense heat, or harmful chemicals is commonly known as a burn (3, 4). These burn injuries can lead to dehydration and a drop in body temperature, creating a favorable environment for germs to thrive. Therefore, ensuring proper healing of such wounds is vital (5).

The bacteria most frequently found in burn injuries are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Stenotrophomonas*, *Enterobacter cloacae*, *Proteus*, and *Escherichia coli* (6). Among these, *Pseudomonas aeruginosa* is the most recognized pathogen associated with wounds. A major hurdle in managing infections in individuals with burns is the growing resistance of these bacteria to antibiotics, along with the rise of strains that are resistant to treatment (7, 8).

Arnebia euchroma, a member of the Boraginaceae family, has a natural range that covers various regions in Asia, including the Himalayas, western Tibet, Iran, and the warmer northern areas of Africa. In the field of traditional medicine, *Arnebia* has been commonly used in the form of vapors or poultices. Extracts from its roots have been utilized for treating wounds, burns, frostbite, and skin inflammations (9). Members of the Boraginaceae family are noted for their roots, which are abundant in shikonin, naphthoquinones, and alkannin (10). These substances and their derivatives display a wide range of biological effects, including wound healing, antifungal, antiviral, anticancer, and anti-inflammatory activities. Traditionally, extracts from *Arnebia* species have been applied topically to disinfect wounds and alleviate inflammation (11).

Recent progress in nanotechnology has resulted in the extensive use of nanoparticles in the field of drug delivery. Among these, liposomes stand out as especially advantageous because of their ability to release drugs steadily and in a controlled manner. Additionally, hydrogels create a three-dimensional framework by absorbing water and bodily fluids, allowing hydrophilic polymers to form a network. Their significant water content and permeability make hydrogels functionally akin to living tissues (12, 13). Given the significant potential of the *Arnebia euchroma* plant, its widespread and diverse use of derived compounds throughout history, its native status and abundance in Iran, and the limitations of antibiotic treatments, the objective of this study is to investigate the efficacy of hyaluronic acid-based hydrogel formulations containing *Arnebia euchroma* nanoliposomes and hydroalcoholic *Arnebia euchroma* extract in accelerating the healing process of second-degree infected burn wounds and reducing infections caused by resistant bacteria.

Materials and Methods

The root of the *Arnebia euchroma* plant was acquired from a nearby store. Following its pulverization, 50 g of the plant material was placed in a Soxhlet apparatus. The compounds present in the plant were extracted using 300 mL of hydroalcoholic solvent. The extraction process was conducted by heating the mixture at 90°C for 15 hours. Subsequently, the solvent was removed by rotary evaporation. The product was an *Arnebia euchroma* extract, which was subsequently concentrated to a 2% solution and stored at 5°C under refrigeration.

Synthesis of *Arnebia euchroma* Liposomes

First, 1.0 g of lecithin was added to 50 mL of deionized water and stirred until dissolved. Following the addition of the plant extract to the aforementioned solution, the mixture was sonicated for 30 minutes. Subsequently, it was homogenized to prepare the nanoliposomes of *Arnebia euchroma*. The resulting liposomes were then stored in the refrigerator at 5°C.

Preparation of Liposomal Hydrogel

In order to prepare the liposomal hydrogel, 10 mL of the nanoliposome was combined with 10 mL of water (forming solution A). Separately, polyethylene glycol and hyaluronic acid were dissolved in 10 mL of water (forming solution B). Solution B was then added to solution A, and the mixture was vigorously stirred for 30 minutes. The resulting liposomal hydrogel was subsequently stored in the refrigerator at 5°C.

Experimental Animals

A total of twenty-four male Wistar albino rats, each weighing between 200 and 250 g and aged 2 to 3 months, were obtained from the animal lab at Kerman University of Medical Sciences in Iran. These rats were randomly divided into six groups, each containing four individuals, leading to a total of eight wounds for every group. Two wounds were created on the right and left sides of the lower back of each rat. The animals were kept in standard polypropylene cages topped with wire mesh, in an environment maintained at a temperature of 21 ± 1°C, under a 12-hour light/dark cycle at the animal care facility of the Veterinary faculty of Shahid Bahonar University of Kerman, Iran. They were given free access to water and pellet food provided by Javaneh

Khorasan Co., located in Mashhad, Iran. The experimental procedures followed the ethical standards set by the Ethics Committee of Kerman University of Medical Sciences. For the surgery, the rats were anesthetized with intraperitoneal injections of ketamine (100 mg/mL, Bremer Pharma GmbH, Germany) and xylazine (20 mg/mL, Alfasan, Holland). The fur on the right and left lower back areas was shaved and prepared according to sterile surgical guidelines (14,15).

Creation of Burn Wounds

A copper rod, shaped like a cylinder and weighing 100 g with a diameter of 1 cm, was fitted with a rubber handle that does not conduct heat. This rod was used to create thermal burns. To heat it, the rod was placed in a bath of boiling water until it reached a temperature of 100°C, which was kept track of using an electronic thermocouple. Once heated, the rod was pressed gently against the skin of the rats, relying only on its own weight, for 10 seconds without applying any extra pressure (15).

Preparation of Bacteria and Infection
Bacterial Strain

Pathogenic bacteria known as *Pseudomonas aeruginosa* (PAO1) were obtained from Kerman University of Medical Sciences in Iran. Initially, these bacteria were cultivated on Nutrient Agar (NA) using a four-quadrant streaking method to ensure their purity. After being incubated for 24 hours at a temperature of 37°C, a single, well-separated colony was chosen and transferred to a liquid Nutrient Broth (NB). This NB culture was then incubated overnight under identical conditions. The concentration of bacteria was measured using a spectrophotometer and adjusted to a standardized McFarland unit of 3×10^8 CFU/mL (16,17).

Infection of Wounds

A quantity of 100 µL of the bacterial solution was introduced into each wound that was 24 hours old. To facilitate deeper bacterial entry and promote infection, small cuts in a crisscross pattern were made on the cleaned wounds with a sterile scalpel before the application of the bacteria. Treatments with topical ointments began 48 hours after the burn injury, on the third day after the initial damage to the wound (18,19).

The Study Groups

The experimental design included six distinct groups: (1) a positive control group treated with zinc oxide (IPOZINC 20%, Tehran, Iran); (2) a treatment group receiving ointment containing nanoliposomes loaded with *Arnebia euchroma* extract; (3) a hydroalcoholic extract group administered ointment containing *Arnebia euchroma* hydroalcoholic extract; (4) a nanoliposome control group treated with blank nanoliposome ointment; (5) a hyaluronic acid control group receiving hyaluronic acid ointment; and (6) a negative control group that received no treatment.

Rats from each test group were administered a targeted treatment with a particular medication for 13 days after the onset of their wound infection. On the 9th and 15th days after the operation, half of the rats from each group were euthanized. Tissue samples from the burned areas, including a 1-cm border of healthy skin, were carefully removed through surgery and swiftly preserved in 10% neutral buffered formalin for at least 48 hours.

Macroscopic Evaluation

The size of the wounds in rats was evaluated on days 1, 3, 5, 7, 9, 13, 14, and 15. To measure the area of the wounds, an L-shaped ruler was used every second day. The ImageJ 1.52V software was used to analyze the wound area using photographic images. Pictures of the wound were taken with a Canon 1Ds Mark II (Japan) digital camera featuring auto flash and a resolution of 17 megapixels, positioned at a fixed distance of 10 cm and oriented perpendicularly to the injury. These images were captured every other day, starting from the day the burns occurred. The percentage of wound contraction was calculated using the formula: [(Initial wound area (A_0) − Wound area at time t (A□)) / A_0] × 100. This measurement reflects the reduction in wound size over time as an indicator of healing progression (20).

Histopathologic Evaluation

After the tissue fixation process, skin samples underwent dehydration through a sequence of progressively concentrated alcohol solutions. They were then cleared with xylene and embedded in paraffin wax, preparing them for histological examination. Thin sections, 5 µm in thickness, were stained with hematoxylin-eosin and Masson's trichrome to enhance visibility under a light microscope. The microscopic analysis of these stained sections facilitated a thorough evaluation of inflammatory reactions, including neutrophil migration, swelling, and increased blood flow, as well as the processes of re-epithelialization, granulation tissue development, collagen accumulation, and the maturation of scars.

A Greenhalgh scoring system, as outlined in Table 1, was adopted to evaluate histopathological parameters (21).

Statistical Analysis

Statistical analysis of the collected data was conducted using IBM SPSS Statistics 27 software. Descriptive statistics, including mean and standard deviation, were calculated to summarize the distribution of the variables under investigation. The normality of the data was assessed using the Kolmogorov-Smirnov test, followed by an evaluation of variance homogeneity using the Levene test. Given the normal distribution of wound area data and the percentage of wound contraction in the study groups, one-way analysis of variance (ANOVA) was employed to determine significant differences between these parameters across the 3rd, 5th, 7th, 9th, 13th, 14th, and 15th days post-burn injury. Tukey's post hoc test was utilized for pairwise comparisons between groups. A statistical significance level of 5% was adopted throughout the analysis. The Kruskal-Wallis test was used in this study to compare the medians of three or more independent groups, as the data were non-normally distributed or ordinal, ensuring a robust non-parametric analysis of group differences.

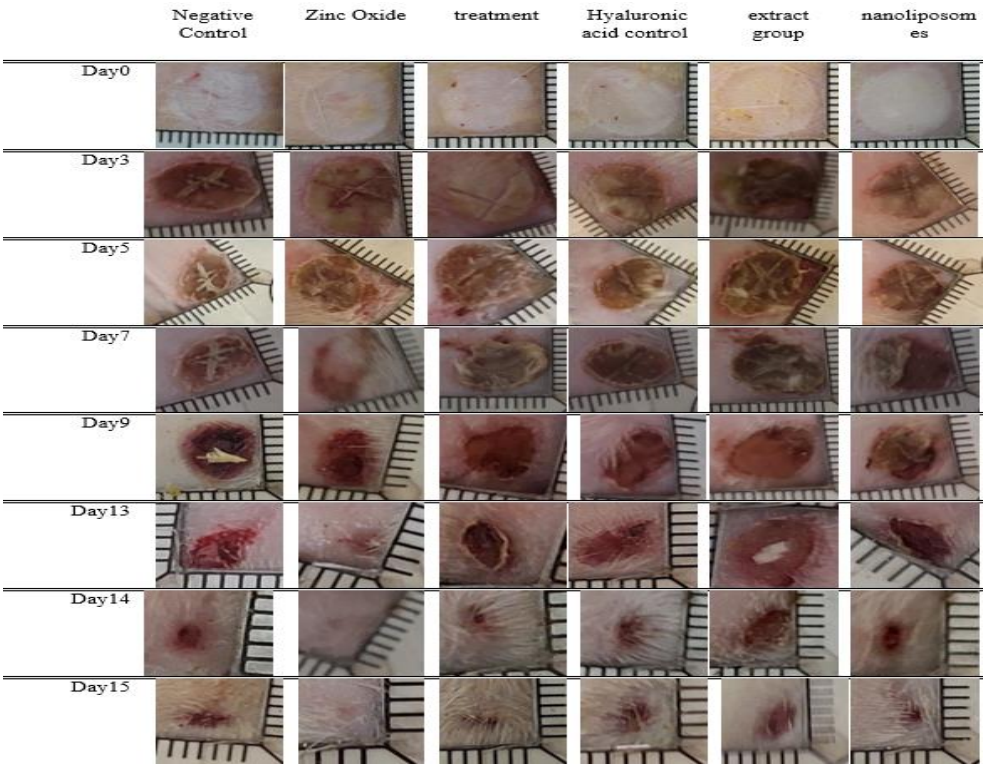

Figure 1. Macroscopic examination of wound contraction in the positive control group, treatment group, extract group, nanoliposome control group, hyaluronic acid control group, and negative control group showing the average wound area on different days after the initial operation.

Results

Characterization of Nano-Metric Liposomes

The Dynamic Light Scattering (DLS) technique is a widely applied method for characterizing the size distribution of nanoparticles, including nanoliposomes (22). Within the scope of this study, DLS was employed to verify the successful synthesis of the nanoliposomes and to evaluate the size distribution of the prepared nanoliposome hydrogel. The acquired data can be analyzed to yield the particle size distribution, represented by the mean diameter or the peak size. In this instance, the mean diameter of the nanoliposomes was determined to be approximately 346 nm.

Comparison of Macroscopic Evaluations

Visual assessments revealed that wounds treated with the ointment containing nanoliposomes loaded with the *Arnebia euchroma* extract group and the positive control group exhibited superior macroscopic healing compared to other groups (Figure 1). These findings were approximately corroborated by statistical analyses of ImageJ software data and histological examinations.

The data and images collected on Day 11 were discarded due to a lack of adherence to proper photographic techniques.

Comparison of Mean Wound Contraction Percentage in Rats

The changes in wound contraction percentage of rats over time and according to the study groups are presented in Figure 2.

The results showed that at the end of the healing period (day 15), the highest mean wound contraction percentage was measured in rats treated with zinc oxide ointment (99.77 ± 0.44%), while the lowest value was observed in the group treated with ointment containing hydroalcoholic extract of the *Arnebia euchroma* (94.92 ± 7.17%).

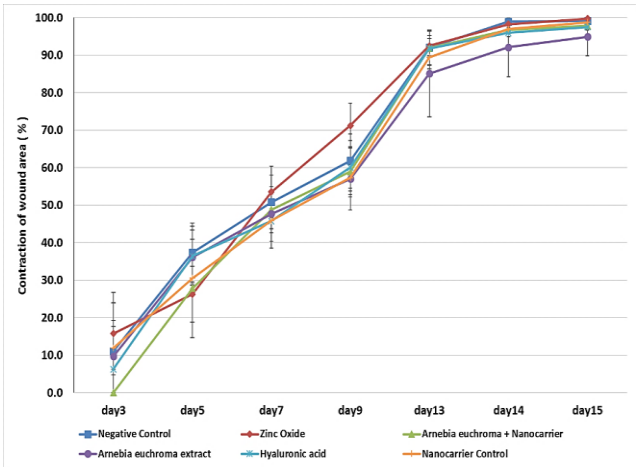

Figure 2. Mean ± standard error of wound contraction percentage changes in rats during the healing period across different study groups.

Table 1. Wound-healing histologic scoring system.[24]

Variable	0	1	2	3
Acute inflammation	None	Scant	Moderate	Abundant
Granulation tissue amount	None	Scant	Moderate	Abundant
Collagen deposition	None	Scant	Moderate	Abundant
Neovascularization	None	Up to five vessels per HPF	6-10 vessels per HPF	More than 10 vessels per HPF

HPF: high-power field.

Statistical Analysis of Histopathological Data

The median (with 25th and 75th percentiles) of qualitative histopathological findings at the 9th and 15th days post-sampling is summarized in Tables 2, 3, and 4.

The Kruskal-Wallis test revealed that on day 9, there was no significant difference in inflammation levels among the study groups ($\chi^2(5) = 8.130$, p = 0.149). However, the same test indicated a significant difference in angiogenesis among the groups on day 9 ($\chi^2(5) = 11.868$, $P = 0.037$). Pairwise comparisons using the Mann-Whitney U test demonstrated a significant difference in angiogenesis between the treatment group and the negative control group on day 9 (p = 0.035). Furthermore, the Kruskal-Wallis test showed a significant difference in granulation tissue formation among the study groups on day 9 ($\chi^2(5) = 12.613$, $P = 0.027$) (Table 2).

The Kruskal-Wallis test revealed a significant difference in collagen deposition among the study groups on day 15 ($\chi^2(5) = 19.457$, $P = 0.002$). Pairwise comparisons using the Mann-Whitney U test showed that the treatment group had significantly higher collagen deposition compared to both the negative control group ($P = 0.002$) and the hydroalcoholic extract group ($P = 0.005$).

Similarly, the Kruskal-Wallis test indicated a significant difference in angiogenesis among the groups on day 15 ($\chi^2(5) = 17.088$, $P = 0.004$). Pairwise comparisons revealed that the treatment group exhibited significantly higher angiogenesis compared to the negative control group ($P = 0.004$).

Furthermore, the Kruskal-Wallis test demonstrated a significant difference in granulation tissue formation among the study groups on day 15 ($\chi^2(5) = 18.539$, $P = 0.002$). Pairwise comparisons showed that the treatment group had significantly higher granulation tissue formation compared to the hydroalcoholic extract group ($P = 0.015$), the nanocarrier control group ($P = 0.015$), and the hyaluronic acid group ($P = 0.012$) (Table 3).

One-way ANOVA revealed no significant difference in the mean percentage of re-epithelialization among the study groups on day 9 ($P > 0.05$). However, on day 15, a significant difference was observed ($F(5,22) = 92.4$, $P = 0.004$). Post hoc Tukey's test on day 15 indicated that the mean percentage of re-epithelialization was significantly higher in the treatment group and the zinc oxide group compared to other groups ($P < 0.05$) (Table 4).

Descriptive Histopathological Evaluation

Histopathological evaluation on day 9 revealed that the negative control group exhibited an initiation of epithelial tissue formation around the burn wound, albeit covering a small percentage of the wound surface. Additionally, high amounts of necrotic tissue were observed at the wound site. At the wound margins and beneath the newly formed, thin epithelial tissue, some granulation tissue with moderate vascularization was evident (Figure 3).

Microscopic examination of the remaining groups and comparison with the negative control group indicated that the best healing process, in terms of re-epithelialization,

granulation tissue formation, and angiogenesis, was observed in both the positive control and treatment groups. The hydroalcoholic extract group demonstrated a better healing process compared to the negative control group; however, it performed less well in some parameters, such as necrotic tissue removal and granulation tissue formation, compared to the positive control and treatment groups. In the nanoliposome and hyaluronic acid control groups, no significant histopathological differences in the healing process were observed compared to the negative control group.

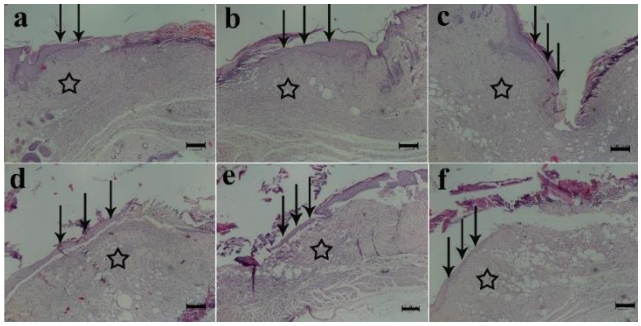

Figure 3. Histopathological evaluation of infected burns on day 9 in different groups. (a) Negative control group: Limited epithelialization has initiated from the wound margin (arrows) with underlying poorly vascularized granulation tissue (asterisk). (b) Positive control group: Well-established epithelialization from the wound margin with abundant and highly vascularized granulation tissue (asterisk). (c) Treatment group: Greater epithelialization compared to the negative control group (arrows) and more abundant and better-quality granulation tissue compared to the negative control group (asterisk). (d) Hydroalcoholic extract group: Well-developed epithelialization (arrows) with moderate granulation tissue in the dermal region (asterisk). (e) Hyaluronic acid control group; (f) Nanoliposome control group: Similar levels of epithelialization and granulation tissue formation compared to the negative control group. (Hematoxylin and eosin stain, ×150).

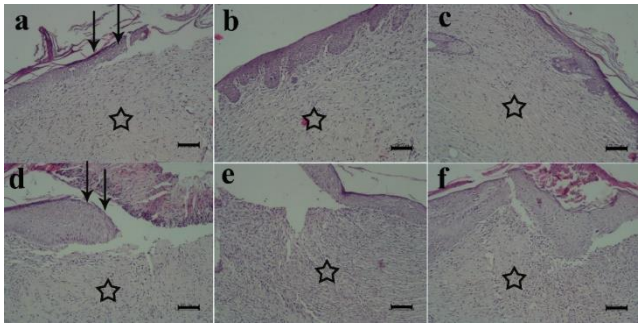

Figure 4. Histopathological evaluation of infected burns on day 15 in different groups. (a) Negative control group: Incomplete epithelialization with immature granulation tissue in the dermis. (b) Positive control group; (c) Treatment group: Complete epithelialization covering the entire wound surface with more mature granulation tissue compared to other groups. (d) Hydroalcoholic extract group: Newly formed epithelium does not cover the entire wound surface, and granulation tissue is relatively mature. (e) Hyaluronic acid control group and (f) Nanoliposome control group: Incomplete epithelialization with immature granulation tissue and high angiogenesis. (Hematoxylin and eosin stain, ×100).

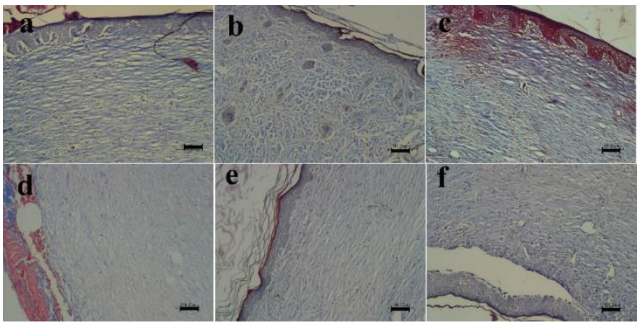

Figure 5. Histopathological evaluation of infected burns on day 15 in different groups. Collagen fibers are stained blue. The positive control group (b) and treatment groups (c) demonstrated the highest collagen production compared to the negative control group. The hydroalcoholic extract group (d), Hyaluronic acid control (e), and Nanoliposome control (f) groups showed lower collagen production. (Masson's trichrome stain, ×100).

On day 15, microscopic evaluation focused on parameters such as percentage of epithelialization, granulation tissue formation, angiogenesis, and collagen production in the repair tissue. The positive control and treatment groups exhibited the best healing process, with complete wound epithelialization and underlying mature, hypocellular, hypovascular granulation tissue with high collagen content. The negative control, nanoliposome control, hyaluronic acid control, and hydroalcoholic extract groups demonstrated the poorest healing process. In many cases, epithelialization was incomplete, and granulation tissue in these groups was relatively immature and hypervascular, with a moderate amount of collagen fibers (Figures 4 and 5).

Discussion

The skin acts as the main interface between the body and the outside world, playing a vital role in protecting against threats. When this barrier is broken, it can allow germs and other harmful agents to enter, putting the overall health of the individual at risk (1).

In innovative transdermal drug delivery methods, the skin plays an essential dual function, serving not just as a protective barrier but also as a key player in the movement of medications. A deep comprehension of the skin's role as a semipermeable barrier has significantly contributed to the improvement and effectiveness of transdermal drug delivery systems (23).

The rising issue of antimicrobial resistance, coupled with the negative consequences of prolonged antibiotic use, has created considerable obstacles in the treatment of infections, especially in cases of burn wounds. This situation

underscores the critical demand for alternative treatment options that carry fewer side effects and offer improved effectiveness. A noteworthy strategy involves the use of natural substances known for their antibacterial qualities. *Arnebia euchroma*, known for its strong components like catechin and flavonoids, has long been used in traditional medicine as a natural treatment for various health issues, ranging from respiratory and digestive problems to rheumatic, heart, and kidney diseases, along with addressing inflammation and infections linked to burns (24).

Table 2. The Effect of liposome nanocarrier containing *Arnebia euchroma* extract on Histological Changes on Day 9 of Healing in Rats. The data were expressed as median (25th percentile, 75th percentile).

Groups	Inflammation	Neovascularization	Formation of granulation tissue
Negative Control	2.5(2.0-3.0) [a]	1.0(1.0-1.75) [a]	2.0(1.25-2.0) [ab]
Zinc Oxide	1.5(1.0-2.75) [a]	2.5(2.0-3.75) [c]	2.5(2.0-3.75) [bc]
Treatment	2.0(2.0-2.0) [a]	2.0(2.0-2.75) [bc]	3.0(2.25-3.0) [c]
Hydroalcoholic extract	1.5(1.0-2.0) [a]	2.0(2.0-2.75) [bc]	2.0(2.0-2.0) [abc]
Hyaluronic acid	2.5(2.0-3.0) [a]	1.5(1.0-2.0) [ab]	2.0(2.0-2.0) [abc]
Nanocarrier Control	2.5(2.0-3.0) [a]	1.5(1.0-2.0) [ab]	1.5(1.0-2.0) [a]
Sig.	0.149	0.037	0.027

There are significant differences between groups with different codes (superscript letters [a, b, c]; $P < 0.05$).

Table 3. The Effect of liposome nanocarrier containing *Arnebia euchroma* extract on Histological changes on Day 15 of Healing in Rats. The data were expressed as median (25th percentile, 75th percentile).

Groups	Collagen deposition	Neovascularization	Formation of granulation tissue
Negative Control	1.5(1.0-2.0) [a]	3.0(3.0-3.0) [c]	3.0(2.25-3.0) [bc]
Zinc Oxide	3.5(3.0-4.0) [d]	1.0(1.0-1.75) [a]	4.0(4.0-4.0) [d]
treatment	3.0(3.0-3.25) [cd]	2.0(1.0-2.0) [ab]	3.5(3.0-4.0) [cd]
Hydroalcoholic extract	2.0(1.25-2.0) [ab]	2.5(2.0-3.0) [bc]	2.0(2.0-2.75) [ab]
Hyaluronic acid	2.5(2.0-3.0) [bc]	3.0(2.0-3.0) [c]	2.0(2.0-3.0) [ab]
Nanocarrier Control	2.0(2.0-2.75) [abc]	2.5(2.0-3.0) [bc]	2.0(2.0-2.75) [ab]
Sig.	0.002	0.004	0.002

There are significant differences between groups with different codes (superscript letters [a, b, c]; P < 0.05).

Table 4. The Effect of liposome nanocarrier containing *Arnebia euchroma* extract Reepithelialization in Day 9-15 of Healing in Rats. The data were expressed as Mean±SEM

Groups	Day	
	9	**15**
Negative Control	15.00 ± 2.04 [a]	95.00 ± 2.88 [bc]
Zinc Oxide	53.75 ± 21.34 [a]	100.00 ± 0.00 [c]
Treatment	31.25 ± 2.39 [a]	100.00 ± 0.00 [c]
Hydroalcoholic extract	47.50 ± 17.50 [a]	82.50 ± 4.33 [a]
Hyaluronic acid	25.00 ± 2.04 [a]	90.00 ± 3.65 [ab]
Nanocarrier Control	21.25 ± 1.25 [a]	90.00 ± 4.08 [ab]
Sig.	0.164	0.004

N= 4 animals in each group. Data are presented as the mean ± SEM. There are significant differences between groups with different codes (superscript letters [a, b, c]; $P < 0.05$ *vs.* carrier control).

Recently, a variety of studies have explored the plant's ability to promote healing, reduce inflammation, and combat burns. Although findings have varied somewhat, most past research has shown that extracts from *Arnebia euchroma* can significantly speed up the healing process of wounds.

The main aim of using nanoparticles as a new type of drug delivery system is to improve the effectiveness of medications while reducing unwanted side effects by altering how the drug behaves in the body. These tiny particles can be made from different substances like polymers and lipids, and their size and form can be customized to meet particular needs (25).

Both nanoparticles and liposomes present notable benefits for drug delivery systems, such as improved effectiveness, lower toxicity levels, and targeted

application. Their adaptability regarding both the materials used and their structural configurations positions them as promising candidates for creating safer and more potent therapeutic solutions. In this research, we focused on assessing the healing properties of *Arnebia euchroma* nanoliposomal extract and hydroalcoholic *Arnebia euchroma* extract on wound recovery. We investigated histopathological alterations in the wound tissues across various treatment groups. Through histopathological techniques, we evaluated several factors, including inflammation, the process of re-epithelialization, blood vessel formation (angiogenesis), and the arrangement of collagen. By performing statistical analysis of the collected data, along with detailed microscopic assessments and overall evaluations of the wounds, we achieved a thorough comparison between the different treatment groups. This approach also contributed to a deeper insight into the therapeutic mechanisms at play.

Analysis of histopathological data up to day nine revealed notable enhancements and advantages in both the treatment group (administered nanoliposomal *Arnebia euchroma* extract) and the positive control group. Additionally, the cohort receiving hydroalcoholic *Arnebia euchroma* extract demonstrated some improvement in various histopathological metrics, including re-epithelialization and diminished inflammation when compared to the other groups. These results are consistent with the observations made by Olczyk et al. (2013), who documented the beneficial effects of *Arnebia euchroma* on wound healing in rabbits (26).

The results align with earlier research, particularly the work of Aliasl et al. (2015), who demonstrated the anti-inflammatory, analgesic, and antibacterial properties of *Arnebia euchroma*. The healing properties of *Arnebia euchroma* extract in treating wounds can be linked to various elements, such as its ability to combat oxidative stress, reduce inflammation, and stimulate fibroblast activity. Key active ingredients in *Arnebia euchroma*, especially shikonin and alkannin, exhibit strong anti-inflammatory characteristics, helping to diminish swelling and speed up the healing process by blocking the production of inflammatory cytokines and enzymes that break down tissue (27). Moreover, these substances encourage the growth of fibroblasts and enhance collagen production, which plays a crucial role in the development of granulation tissue and the process of epithelial healing.

The hydroalcoholic extract of *Arnebia euchroma* showed better results in specific pathological aspects, particularly in re-epithelialization by the ninth day, when compared to the nanoliposomal extract. This apparent benefit can be linked to the ethanol content in the hydroalcoholic extract. Ethanol acts as a strong disinfectant, helping to manage surface wound infections effectively, thus creating a more favorable environment for healing. On the other hand, the nanoliposomal formulation presents several benefits, including more precise delivery to the infected area, quicker bacterial removal, and a decrease in surface wound infections. These observations are consistent with the study conducted by Jarahi and his team in 2008, which highlighted the significant role of hydroalcoholic derivatives during the initial phases of burn wound recovery (28).

On the fifteenth day of observation, both the group receiving nanoliposomal *Arnebia euchroma* extract and the positive control group exhibited notable enhancements in wound healing measures, according to histopathological and macroscopic assessments. In contrast, although the group treated with hydroalcoholic *Arnebia euchroma* extract also displayed some degree of wound healing, its effectiveness fell short compared to the others in specific criteria.

Extended use of hydroalcoholic substances has been demonstrated to slow down the healing of wounds and interfere with different phases of the repair process due to the oxidative properties of ethanol. In this current investigation, from days nine to fifteen after the burn, when the wound infection was well managed, the use of the hydroalcoholic extract led to dryness in the wound area and hindered the healing mechanisms. This observation aligns with Brånemark's research from 1967, which highlighted the detrimental effects of alcohol derivatives on wound recovery and the resulting tissue damage (29). Furthermore, research conducted by Radek in 2005 revealed that alcohol and its byproducts can greatly hinder the healing of wounds. Both ongoing and short-term exposure to alcohol interferes with essential processes involved in wound recovery, leading to slower healing, reduced collagen production, and compromised blood vessel formation. Importantly, these negative effects were noted even when typical amounts of vascular growth factors, like VEGF and FGF2, were present, indicating that alcohol has a direct influence on the functioning of endothelial cells (30).

One possible explanation for the minimal variation observed in the group receiving 2% hydroalcoholic *Arnebia euchroma* extract could be due to an inadequate concentration of the extract. Prior research indicates that the effectiveness of hydroalcoholic *Arnebia euchroma* extract is heavily dependent on its concentration, highlighting the importance of a therapeutic range for any medication. A study by Tahmasebi et al. (2013) revealed that both 1% and 10% concentrations of hydroalcoholic *Arnebia euchroma* extract exhibited only a limited therapeutic benefit in

promoting wound healing (31). However, in the present study, the range of concentrations investigated may not have been sufficient to determine the optimal hydroalcoholic-based concentration. Therefore, it is recommended that future studies investigate a broader range of concentrations.

The findings of this study highlight that the group receiving treatment with nanoliposomal *Arnebia euchroma* extract significantly outperformed the other groups in promoting and speeding up the healing of wounds. A key factor behind this advantage lies in the distinctive characteristics of nanoparticles and nanoliposomal systems. These systems improve the effectiveness of the treatment by facilitating the controlled release of bioactive substances, which allows them to remain active at the wound site for a longer duration. This observation aligns with Lee's research conducted in 2014, which underscored the important role of nanoparticles in achieving controlled release and enhancing the advanced properties of medications (32).

A significant factor contributing to this advantage is the ability of this structure to create a protective setting, which boosts the stability of the drug's active components in its environment and helps prevent their breakdown. As a result, the concentration of the drug's functional elements remains effective at the wound site for an extended duration, thus enhancing its healing properties. The results of the current research align with those reported by Hemati et al. (2023), who indicated that liposomal structures based on hydrogels can serve as effective drug delivery systems, thanks to their enhanced compatibility with biological tissues, improved strength, and controlled release of medication (33).

A key factor contributing to the enhanced wound healing seen in the group treated with the nanoliposomal extract of *Arnebia euchroma* is the combined action of the drug components within the hydrogel-based nanoliposomal system. This innovative structure fosters a damp and shielded atmosphere, which is essential for effective wound recovery. Thirumaleshwar's research in 2012 further validated that liposomal hydrogels form a protective layer, preventing wound infection and maintaining a moist environment that promotes healing (34).

Moreover, nanoliposomes, due to their ability to combat oxidation, shield bioactive substances from breaking down by proteolytic enzymes present in the wound area. Furthermore, the improved ability of nanoliposomes to penetrate cells and concentrate drugs in specific locations establishes an ideal setting for drug molecules to enter cells and speed up the healing process. Essentially, nanoliposomes play a crucial role in promoting wound healing by boosting the stability, availability, and effectiveness of bioactive substances.

To sum up, utilizing nanoliposomes made from hyaluronic acid hydrogel to administer a 2% extract of *Arnebia euchroma* on second-degree burn injuries infected with Pseudomonas aeruginosa led to notable advancements in the healing speed and overall quality of the wounds. This evidence bolsters the use of nanoliposomal *Arnebia euchroma* extract in clinical settings for treating infected skin burns.

Acknowledgements

We thank all our colleagues who participated in this project and helped us.

Authors' Contributions

Mohammad Mahdi Molaei, Ehsanollah Sakhaee, Reza Kheirandish, and Pourya Mohammadi conceived and planned the experiments. **Mohammadmahdi Alinaghizadeh, Fatemeh Heydari, and Reza Molaei** conducted the experiments, and analyzed the data. **Fateme Mohebbi Pourkani** wrote the initial draft of the manuscript. **Ehsanollah Sakhaee** took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability

All data generated or analyzed during this study are presented within this published article. No additional datasets were used or are available from other sources.

Ethical Approval

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

Conflict of Interest

The authors declare there is no conflict of interest.

Consent for Publication

Not applicable.

Funding

This study was not supported by any funding.

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