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Original Article

The Effect of Liposome Nanocarrier Containing *Scrophularia striata* Extract on Burn Wound Healing in Rats

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ABSTRACT

The present study was conducted to evaluate the wound healing effects of liposome nanocarrier containing *Scrophularia striata* extract. Seventy-two male Wistar albino rats were divided into 6 groups of 12 animals each. Rats were anesthetized, and dorsum shaved. A 100 g cylindrical copper rod of 1cm diameter was heated up to 100° C. It was placed on the skin without any pressure. Histopathological evaluation and the macroscopic size processing and analysis of the burn were employed to study the potential of wound healing in study groups. The study groups were: positive control group (zinc oxide treatment), treatment group (ointment containing nanoliposomes loaded with extract *Scrophularia striata*), extract group (ointment containing hydroalcoholic extract *Scrophularia striata*), control group of nanoliposomes (ointment containing Nano liposomes), Eucerin control group (ointment containing Eucerin), negative control group (no treatment). On the 5th, 10th, and 15th days after the initial operation, samples were taken from 4 rats of each group. The results showed that loaded nanoliposomes had a nanometric size and spherical morphology. Also, the wound size of the treatment group was smaller than other groups and had the best effect on repairing wound healing. Also, in other groups, positive control had a better effect on wound repair than extract, Eucerin, nano, and negative groups. According to the highest re-epithelialization, granulation tissue formation, and the lowest necrotic tissue, we could suggest that the treatment group had the best healing effect among other groups.

Introduction

The skin is the largest organ of the body and the first defense barrier.[1] Any loss of integrity of the skin layers, including the dermis and epidermis, is called a wound.[2] Burns are coagulation necrosis of body organs caused by thermal energy. Burn wounds are one of the most common wounds in chemical, thermal, or electrical burns that cause skin injuries or mucous membranes.[3] Burn lesions lead to dehydration and hypothermia and

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are a suitable environment for the growth of microorganisms. Hence, the healing process of these wounds is vital.[4] Wound healing is a natural healing process in the body which stages are: inflammation, proliferation (including granulation tissue formation and re-epithelialization), and remodeling. Wound location, wound size, nutrition, and age are all effective in wound healing. So far, a lot of research has been done on the use of traditional and chemical drugs. Successful treatment of burns is made possible by a better understanding the pathophysiology of burn injuries and advances in medical technology and surgical techniques.[5,6]

Scrophularia is a large genus of the Scrophulariaceae family. The Scrophularia species have been utilized in traditional medicine since ancient times. The most significant one of those is *Scrophularia striata* as a perennial herbaceous plant that is broadly applied in Iranian folk medicine for infectious and inflammatory diseases. This species has various names in different regions, such as Mashineh, Teshneh Dari, Benj Ghan, and Benjek. People in the western areas of Iran claim that this plant can treat various diseases such as otitis, hemorrhoids, conjunctivitis, colds, gastritis, infectious wounds, and burns. All parts of this plant have been applied in traditional medicine. The aqueous extract of its aerial parts is utilized mainly for the treatment of second and third-degree burns. Its topical utilization remarkably accelerates the healing of burn damage with minimum scar formation.[7,8]

Nanotechnology has been prosperous in obtaining approaches to incorporate antibiotics in nanostructures to treat localized wounds and preventing systemic drug exposure. Many advances have been made in nanotechnology in the formulation of wound dressing and tissue engineering to treat infections of burn wounds.[9,10] Liposomes have closed vesicular structures as closed and continuous bilayer structures mainly composed of phospholipid and/or lipid molecules. Promising nanocarriers for topical drug delivery are liposomes that enclose the water chamber and enable the transfer of molecules with different properties (lipophilic, hydrophilic, and amphipathic), so keeping the functionality and stability of encapsulated materials. They are biocompatible, biodegradable, and non-toxic able to preserve encapsulated drug and treatment agents. Liposomes have been reported to have the most biological cell characteristics needed for a suitable drug delivery system in general and specifically for growth factors for wound treatment.[11,12]

So, in this study, we decided to evaluate the effect of liposome nanocarrier containing *Scrophularia striata* extract on burn wound healing in rats.

Materials and Methods

Preparation of Scrophularia striata Liposomal Ointment

The powder of *Scrophularia striata* plant (75 g) was added to the soxhlet after adding 300 mL ethanol (50%) and then heated at 90° C for 10 h. The solvent was evaporated by a rotary evaporator. The extract was stored in the refrigerator at 5° C.[13] For synthesis of *Scrophularia striata* liposomes, first, 1.0 g of lecithin was added to the 50 ml of deionized water and stirred to dissolve it. Then extract of *Scrophularia striata* was added to the above solution. After that, the mixture was sonicated for 30 min. In the next step, it was mixed with a homogenizer. Nano liposome of *Scrophularia striata* was prepared and then stored in the refrigerator at 10° C.[14] To prepare the liposomal ointment, first, the nanoliposome (10 ml) was added to the Eucerin (20 g). This mixture was stirred strongly for 30 min. The obtained liposomal ointment was stored in the refrigerator at 10° C.[15]

Size and Morphology of Liposomes

Using the Zetasizer Nano ZS, the particle size (nm), zeta potential (mV), and polydispersity index (PDI) of the liposomes were determined by the DLS method (Malvern, Helix, UK). At a temperature of 25° C, the analysis was carried out with a He-Ne laser (wavelength of 633 nm) and a detector angle of 90°. Before analysis, samples were diluted in deionized water with a dilution factor of 1:20. The measurements of size and zeta were done in triplicate, and the results are expressed as Mean ± SD (nm and mV, respectively).

The morphology of the liposomes (after sonication and filtration procedure) was characterized using transmission electron microscopy (EM10C, Zeiss, Germany) operating at 120 kV. For this aim the modified method was used, to lower the concentration of vesicles, the liposomes were diluted with deionized water at a dilution rate of 1:20. On a copper grid, a drop of diluted sample was deposited and allowed to air dry at room temperature. Phosphotungstic acid was used to stain the grid. Filter paper was used to remove excess liquid, which was then dried in a desiccator. The grid was then installed in the device, and images at various magnifications were obtained.[16]

Experimental Animals

72 male Wistar albino rats (200-250 g, 2-3 months old) were obtained from the animal laboratory of Kerman University of Medical Sciences, Kerman, Iran. The rats were randomly divided into six groups of twelve animals. They were housed in standard polypropylene cages with wire mesh top, at 21 ± 1° C in a 12h/12h dark-light cycle in the laboratory animal care center at the Veterinary Medicine School of Shahid Bahonar University of Kerman, Iran. Animals had free access to water and pellet food (Javaneh Khorasan Co, Mashhad, Iran). The experimental protocols were conducted by the ethics committee of the Kerman University of Medical Sciences guidelines. The rats were anesthetized with intra-peritoneal ketamine (100 mg/ml, Bremer Pharma GmbH, Germany) and xylazine (20 mg/mL, Alfasan, Holland). The dorsal surface of the rats was shaved. They were prepared according to the principles of surgical asepsis.[17,18]

Creation of Burn Wounds

A 100 g cylindrical copper rod of 1cm diameter with an exaction rubber handle was used to inflict burns. The rod was plunging in a flask of boiling water and heated up to 100° C. An electronic thermocouple was used to measure the temperature. The heated rod was placed perpendicular to the skin, resting on its own weight for 10 seconds without any pressure.[18]

Rats in each group were treated topically with a specific drug for 15th days after induction of anesthesia and burns. On the 5th, 10th, and 15th days after the initial operation, 4 rats of each group were euthanized, and the burned samples with a margin 1 cm of healthy skin by surgical technique were taken and immediately fixed in the 10% neutral buffered formalin for minimal time of 48 h.

The study groups are positive control group (zinc oxide, IPOZINC 25%, Tehran, Iran), treatment group (ointment containing Nano liposomes loaded with extract *Scrophularia striata*), extract group (ointment containing hydroalcoholic extract *Scrophularia striata*), control group of nanoliposomes (ointment containing Nano liposomes), Eucerin control group (ointment containing Eucerin), negative control group (no treatment).

Geometric Assessment

The size of the burn wound was checked once every two days using an L-shaped ruler and photographed by a digital camera and then examined by Image j software. It was performed using a digital camera (Canon 1DS, Japan) with auto flash and 17-megapixel resolution.[19]

Histopathologic Evaluation

After skin samples fixation, the fixed samples were dehydrated in the different graded alcohols, cleared in xylene and embedded in paraffin wax. Sections in 5 µm thickness were stained with hematoxylin-eosin and Masson's trichrome studied under a light microscope. Taken photomicrographs were investigated to determine inflammation (infiltration of neutrophils, edema and hyperemia), re-epithelialization, granulation tissue formation, collagen deposition, and scar maturation based on histological scoring system described by Hazrati *et. al.*[20]

Statistical Analysis

Statistical analysis of the obtained data was performed using IBM SPSS Statistics 25 software (SPSS25). Descriptive findings of the studied variables, including indicators such as mean and standard deviation, were calculated and reported. The normality of the data was evaluated using the Kolmograph-Smirnov test. Then the homogeneity of variance of the data was assessed by the Leven test. In the next step, according to the normal distribution of wound area data and also the percentage of wound contraction in the study groups, using one-way analysis of variance and Tukey *post hoc* test, the difference between the mean wound area and the percentage of wound contraction during 3rd, 6th, 9th, 12th, and 15th days after burn wounds were evaluated. In all stages of the analysis, the allowable error for rejecting the null hypothesis was considered 5%.

Results

Characterization of Nano-Metric Liposomes

The application of liposomes as plant-derived material carrier systems has been broadly studied. However, the principal difficulty associated with the use of liposomes as carriers of these substances is inadequate delivery to the desired site.[21,22] In addition, preparing plant-derived polyphenol-loaded liposomes with great encapsulation effectiveness may not be simple, as variable interactions between these substances and bilayer lipids can happen. To overcome these difficulties, the researchers focused on

manipulating lipid membrane components in liposomes.[22,23] In this research, we investigated the pharmacological effect of *Scrophularia striata* extract into neutral cationic nanoliposomes on burn wound infection in in-vitro and in-vivo. Dynamic light scattering (DLS) analysis was used to confirm the synthesized nanoparticles. Therefore, the size distribution of *Scrophularia striata* nanoliposomes was obtained. The average diameter of nanoliposomes was about 68 nm (Figure 1A). Also, TEM image of liposome in Eucerin medium was showed in Figure 1B. As shown in this figure, liposomes have a spherical morphology with an average size of 65 nm. Also, liposome have a good distribution in Eucerin medium.

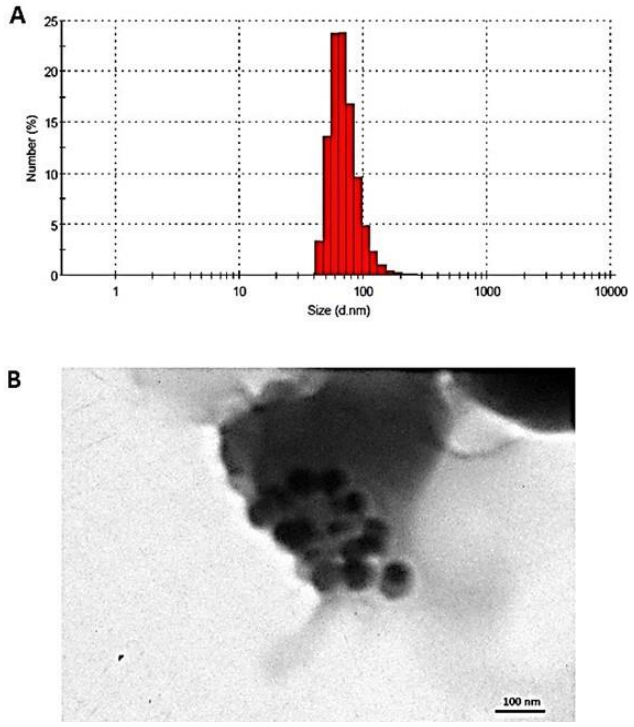

Figure 1. Size distribution and morphology observation of *Scrophularia striata* nanoliposome by dynamic light scattering (DLS) (A), and transmission electron microscopy (TEM) (B).

Comparison of Mean Wound Area in Rats

The photographs showed that the wounds of the treatment group had a better macroscopic improvement than the other groups (Figure 2), which is confirmed by the results of the analysis of statistical data from the Imaging J software and histopathology. Wound area in rats during the healing period according to the type of compounds are shown in Figure 3. Wound area in rats during the healing period according to the type of compounds studied. The results show that at the end of the healing period (15th day), the lowest mean wound area was measured in rats treated with an ointment containing nanoliposomes loaded with *Scrophularia striata* extract (0.019 ± 0.01 mm^2) and the highest value to the negative control group (normal saline based on Eucerin) (0.208 ± 0.02 mm^2). Also, in the positive control group, the mean wound area was higher than the treatment group and was (0.088 ± 0.02 mm^2).

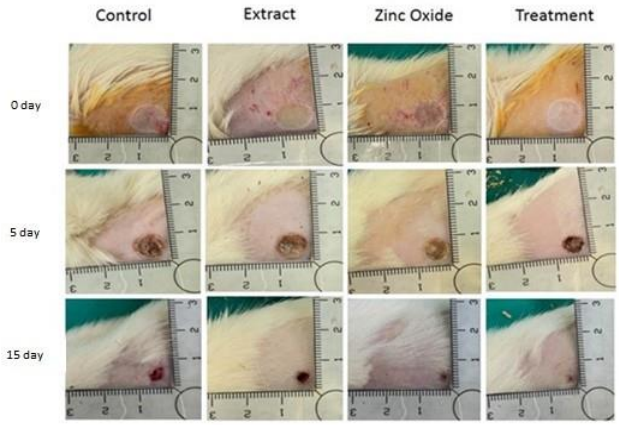

Figure 2. Macroscopic examination of wound contraction on the treatment group (ointment containing nanoliposomes loaded with *Scrophularia striata* extract), positive control group (zinc oxide ointment), control group of nanoliposomes (ointment containing nanoliposomes), extract group (ointment containing hydroalcoholic extract of *Scrophularia striata*), Eucerin control group (ointment containing Eucerin) and negative control group (normal saline based on Eucerin) on the average wound area on 0, 5th, and 15th days after the initial operation.

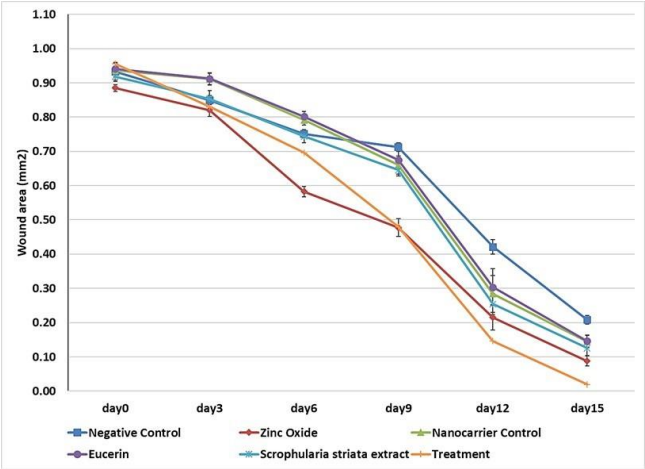

Figure 3. Wound area during the healing period on the treatment group (ointment containing nanoliposomes loaded with *Scrophularia striata* extract), positive control group (zinc oxide ointment), control group of nanoliposomes (ointment containing nanoliposomes), extract group (ointment containing hydroalcoholic extract of *Scrophularia striata*), Eucerin control group (ointment containing Eucerin) and negative control group (normal saline based on Eucerin) on 0, 3rd ,6th , 9th, 12th, and 15th days after the initial operation.

The results of one-way analysis of variance and Tukey's complimentary test to evaluate the difference between the mean wound areas in rats during the recovery period between the studied compounds are presented in Table 1. The results showed that on the third day, the mean wound area in rats in the treatment group and positive control group was significantly lower than the control groups of nanoliposome, control of Eucerin ($p < 0.05$), but no significant difference was observed between negative control and extract treatment groups. On the sixth day, the mean wound area in rats of the positive control group was significantly lower than the treatment, extract, nanoliposome control, and Eucerin control groups ($p < 0.05$), But the treatment group was not significantly different from the negative control group and the extract group. On the ninth day, the mean wound area in rats in the treatment group and positive control group was significantly lower than the control groups of nanoliposome, Eucerin control, negative control, and extract ($p < 0.05$). On the 12th day, the mean wound

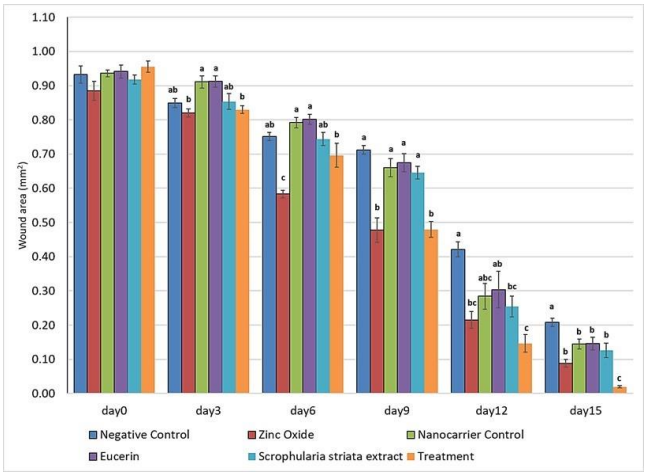

Figure 4. Comparison of the mean wound area in rats on the treatment group (ointment containing nanoliposomes loaded with *Scrophularia striata* extract), positive control group (zinc oxide ointment), control group of nanoliposomes (ointment containing nanoliposomes), extract group (ointment containing hydroalcoholic extract of *Scrophularia striata*), Eucerin control group (ointment containing Eucerin) and negative control group (normal saline based on Eucerin) on 0, 3rd ,6th , 9th, 12th, and 15th days after the initial operation.

Table 1. Effect of liposome nanocarrier containing *Scrophularia striata* extract on burn wound area (mm2) on various days of healing in Rats.

Groups	Wound area (mm²) (Mean ± SD)					
	0st day	3rd day	6th day	9th day	12th day	15th day
Negative Control	0.932 ± 0.05	0.849±0.03 ab	0.751 ± 0.02 ab	0.712 ± 0.02 a	0.421 ± 0.04 a	0.208 ± 0.02 a
Positive control	0.884 ± 0.06	0.819±0.02 b	0.582 ± 0.02 c	0.477 ± 0.07 b	0.215 ± 0.05 bc	0.088 ± 0.02 b
Nanoliposomes	0.936 ± 0.02	0.910±0.03 a	0.791 ± 0.03 a	0.660 ± 0.05 a	0.284 ± 0.07 abc	0.144 ± 0.03 b
Eucerin	0.941 ± 0.04	0.912±0.03 a	0.801 ± 0.03 a	0.675 ± 0.05 a	0.303 ± 0.10 ab	0.146 ± 0.04 b
Extract	0.914 ± 0.03	0.853±0.05 ab	0.743 ± 0.04 ab	0.645 ± 0.04 a	0.254 ± 0.06 bc	0.125 ± 0.04 b
Treatment	0.955 ± 0.03	0.829±0.02 b	0.696 ± 0.07 b	0.479 ± 0.05 b	0.146 ± 0.05 c	0.019 ± 0.01 c

n= 4 animals in each group. Data are presented as the mean ± SD. There are significant differences between groups with different letters (superscript letters a, b, c; $p < 0.05$ vs. carrier control).

Table 2. Effect of liposome nanocarrier containing *Scrophularia striata* extract on contraction of wound area (%) on various days of healing in Rats.

Groups	Contraction of wound area (%) (Mean ± SD)				
	3rd day	6th day	9th day	12th day	15th day
Negative Control	8.69 ± 5.73 ab	19.30 ±3.55 bc	23.51 ± 2.87 b	54.79 ± 4.10 c	77.70 ± 1.57 c
Positive control	7.18 ± 3.61 ab	33.92 ± 4.91 a	46.10 ± 6.65 a	75.58 ± 5.79 ab	90.01 ± 2.58 b
Nanoliposomes	2.74 ± 1.90 b	15.41 ± 3.34 c	29.42 ± 6.15 b	69.60 ± 8.20 b	84.528 ± 3.32 b
Eucerin	3.06 ± 1.48 b	14.80 ± 1.76 c	28.31 ± 3.78 b	67.82 ± 11.03 bc	84.53 ± 3.53 b
Extract	7.02 ± 4.28 ab	18.93 ± 4.70 bc	29.58 ± 5.41 b	72.44 ± 5.74 ab	86.38 ± 4.14 b
Treatment	13.14 ± 1.36 a	27.22 ±5.47 ab	49.84 ± 3.87 a	84.80 ± 5.04 a	97.97 ± 0.76 a

n= 4 animals in each group. Data are presented as the mean ± SD. There are significant differences between groups with different letters (superscript letters a, b, c; $p < 0.05$ vs. carrier control).

area in rats in the treatment group was significantly lower than the negative control and Eucerin control groups ($p < 0.05$), but no significant difference was observed between the treatment group with positive control group, extract, and nanoliposome control). On the 15th day, the mean wound area in the rats of the treatment group was significantly lower than the positive control group, extract nanoliposome control, eucerin control, and negative control ($p < 0.05$) (Figure 4).

Comparison of the mean percentage of wound contraction in rats

Changes in the rate of wound contraction in rats during the measurement times and according to the studied compounds are presented in Figure 5. The results showed that at the end of the healing period (15th day), the highest mean percentage of wound contraction was measured in rats treated with an ointment containing nanoliposomes loaded with *Scrophularia striata* extract (97.97 ± 0.76). The lowest value belongs to the negative control group (normal saline based on Eucerin) (77.70 ± 1.57). In the group treated with positive control group, the average wound contraction percentage was lower than in the treatment group (90.01 ± 2.58).

The one-way analysis of variance and Tukey's supplementary test to investigate the difference between the mean percentages of wound contraction in rats during the recovery period between the studied compounds are presented in Table 2. The results showed that on the 3rd day, the mean percentage of wound contraction in rats in the treatment group was significantly higher than the nanoliposome control and Eucerin control groups ($p < 0.05$); however, no significant difference was observed between the mean percentage of wound contraction in the group treated with positive control groups, negative control groups, and extract groups. On the 6th day, the mean wound contraction percentage in rats of positive control group and treatment group was significantly higher than nanoliposome control and Eucerin control groups ($p < .05$); however, no significant difference was observed between the mean wound contraction percentage of the treatment group with the negative control group and the extract group. On the 9th day, the mean percentage of wound contraction in the treatment group and positive control group was significantly higher than the control group of nanoliposome, Eucerin control, negative control, and extract ($p < 0.05$) groups. On the 12th day, the mean percentage of wound contraction in rats in the treatment group was significantly higher than the negative control, nanoliposome control, and Eucerin control groups ($p < 0.05$). However, no significant difference was observed between the mean wound contraction of the treatment group with positive control group and extract groups. On the 15th day, the mean percentage of wound contraction in rats in the treatment group was significantly higher than the groups of positive control group, extract, nanoliposome

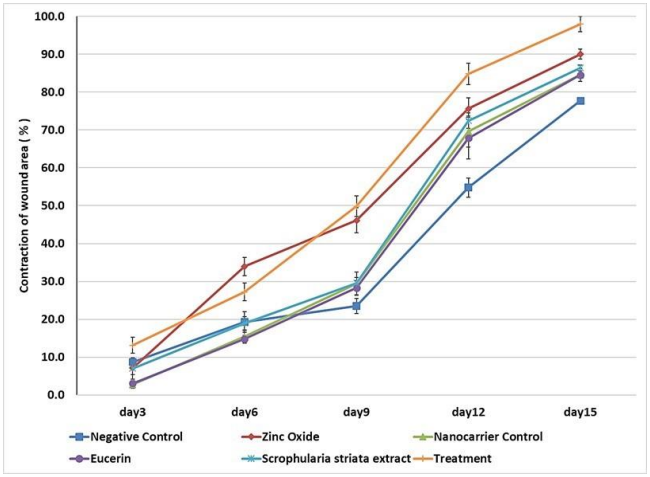

Figure 5. Percentage of wound contraction (Mean ± SE) on the treatment group (ointment containing nanoliposomes loaded with *Scrophularia striata* extract), positive control group (zinc oxide ointment), control group of nanoliposomes (ointment containing nanoliposomes), extract group (ointment containing hydroalcoholic extract of *Scrophularia striata*), Eucerin control group (ointment containing Eucerin) and negative control group (normal saline based on Eucerin) on , 3rd ,6th , 9th, 12th, and 15th days after the initial operation.

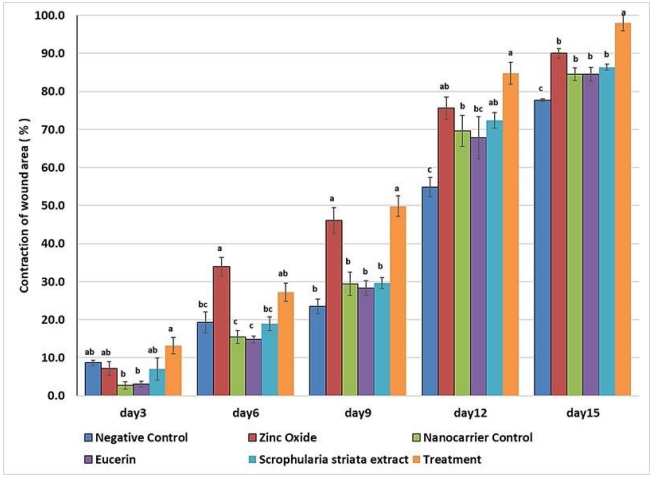

Figure 6. Comparison of the mean percentage of wound contraction in rats according to the type of compounds studied by Tukey method at 95% probability level of columns with the same letters every day is not significantly different. The results of the Kruskal-Wallis test showed that there was a significant difference in the amount of acute inflammation on day 5 between the study groups ($p = 0.001$).

control, Eucerin control, and negative control ($p < 0.05$) (Figure 6).

Qualitative data are presented in the Tables 3-5 as median (25th percentile and 75th percentile). Two by two comparison with Mann-Whitney method showed in Table 3 on 5th day in the amount of acute inflammation between the treatment group with the negative control group ($p = 0.001$), nanocarrier control ($p = 0.002$), and Eucerin ($p = 0.002$) statistically significant ($p < 0.01$). The results of the Kruskal-Wallis test showed that on 5th day, there was a significant difference in the amount of granulation tissue between the studied groups ($\chi2$ (5) = 16.177, $p = 0.006$). Two by two compared with the Mann-Whitney method showed that on 5th day, the amount of granulation tissue between treatment group and negative control group ($p = 0.002$), nanocarrier control ($p = 0.002$), and Eucerin ($p = 0.002$) was statistically significant ($p < 0.01$). There was also a significant difference in granulation tissue between the treatment group and the positive control group ($p = 0.022$) and extract ($p = 0.015$). The results of the Kruskal-Wallis test showed that on 5th day, there was a significant difference in the amount of re-epithelialization between the studied groups ($\chi2$ (5) = 18.524, $p = 0.002$). Two by two, compared with the Mann-Whitney method on 5th day, showed a statistically significant difference in the amount of re-epithelialization between the treatment group and the negative control group ($p = 0.001$), nanocarrier control ($p = 0.001$), and Eucerin ($p = 0.003$). There was also a significant difference in the amount of re-epithelialization between the treatment group and the positive control group ($p = 0.017$) and extract ($p = 0.019$). The results of the Kruskal-Wallis test on 5th day showed a significant difference in the amount of neovascularization between the studied groups ($\chi2$ (5) = 17.638, $p = 0.003$). Two by two, compared with the Mann-Whitney method on 5th day showed a statistically significant difference in the amount of neovascularization between the treatment group and the negative control group ($p = 0.001$), nanocarrier control ($p = 0.002$), and Eucerin ($p = 0.002$). There was also a significant difference in the amount of neovascularization between the treatment group and the positive control group ($p = 0.015$) and extract ($p = 0.015$).

The results of the Kruskal-Wallis test on 10th day (Table 4) showed a significant difference between the studied groups in the amount of acute inflammation ($\chi2$ (5) = 18.356, p = 0.003). Two by two comparison with Mann-Whitney method showed on 10th day in the amount of acute inflammation between the treatment group with the group of negative control ($p = 0.001$), nanocarrier control ($p = 0.001$), and Eucerin ($p = 0.003$) statistically significant at the level ($p < 0.01$). There was also a significant difference in the amount of acute inflammation between the treatment group and the positive control group ($p = 0.011$) and ($p = 0.011$) extract. The results of the Kruskal-Wallis test showed that on 10th day, there was a significant difference in the amount of granulation tissue between the studied groups ($\chi2$ (5) = 16.967, $p = 0.005$). Two by two comparison with Mann-Whitney method showed on 10th day in amount of granulation tissue value between treatment group with the negative control group ($p = 0.001$), nanocarrier control ($p = 0.002$), Eucerin ($p = 0.007$) statistically significant there were levels ($p <0.01$). There was also a considerable difference between the treatment group and the positive control group ($p = 0.013$) and extract ($p = 0.011$) in the amount of granulation tissue. The results of the Kruskal-Wallis test showed that on 10th day, there was a significant difference in the amount of collagen deposition between the studied groups ($\chi2(5) = 17.365$, p = 0.004).

Table 3. Scored results of various histopathological parameter in different experiment groups on the 5th day of healing in rats. The data were expressed as median (25th percentile, 75th percentile).

Groups	Acute inflammation	Amount of GT	Reepithelialization	Neovascularization
Negative Control	4(4.0-4.0) ^{c}	0.0(0.0-0.75) ^{a}	0.0(0.0-3.75) ^{a}	0.0(0.0-0.0) ^{a}
Positive control	2.0(2.0-2.75) ^{b}	1.0(1.0-1.75) ^{a}	10.0(6.25-10.0) ^{b}	1.0(1.0-1.0) ^{b}
Nanoliposomes	4.0(3.25-4.0) ^{c}	0.0(0.0-0.75) ^{a}	0.0(0.0-3.75) ^{a}	0.0(0.0-0.75) ^{ab}
Eucerin	4.0(3.25-4.0) ^{c}	0.0(0.0-0.75) ^{a}	2.5(0.0-5.0) ^{ab}	0.0(0.0-0.75) ^{ab}
Extract	3.0(2.25-3.0) ^{b}	1.0(0.25-1.0) ^{a}	7.5(5.0-10.0) ^{ab}	1.0(0.25-1.0) ^{ab}
Treatment	0.5(0.0-1.0) ^{a}	3.0(2.0-3.0) ^{b}	30(22.5-33.75) ^{c}	3(2.25-3.0) ^{c}

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

Table 4. Scored results of various histopathological parameter in different experiment groups on the 10th day of healing in Rats. The data were expressed as median (25th percentile, 75th percentile).

Groups	Acute inflammation	Amount of GT	Collagen deposition	Reepithelialization	Neovascularization
Negative Control	3(3.0-3.75) [c]	0.0(0.0-0.75) [a]	0.5(0.0-1.0) [a]	12.5(10.0-18.75) [a]	0.0(0.0-0.75) [a]
Positive control	2.0(1.25-2.0) [b]	1.5(1.0-2.0) [b]	2.0(1.25-2.0) [b]	35.0(35.0-38.75) [c]	2.0(1.25-2.0) [b]
Nanoliposomes	3.0(3.0-3.75) [c]	0.5(0.0-1.0) [ab]	1.0(0.25-1.0) [ab]	17.5(15.0-23.75) [ab]	0.5(0.0-1.0) [a]
Eucerin	3.0(2.25-3.75) [bc]	1.0(0.25-1.0) [ab]	0.5(0.0-1.0) [a]	17.5(11.25-20.0) [ab]	0.5(0.0-1.0) [ab]
Extract	2.0(2.0-2.75) [bc]	1.0(1.0-1.75) [ab]	1.5(1.0-2.0) [ab]	30.0(22.5-33.75) [b]	1.5(1.0-2.0) [ab]
Treatment	0.0(0.0-0.0)[a]	4.0(4.0-4.0) [c]	4(3.25-4.0) [c]	80.0(76.25-83.75) [d]	4(3.25-4.0) [c]

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

Table 5. Scored results of various histopathological parameter in different experiment groups on the 15th day of healing in Rats. The data were expressed as median (25th percentile, 75th percentile).

Groups	Acute inflammation	GT Maturation	Collagen deposition	Reepithelialization
Negative Control	2.0(1.25-2.0) [b]	0.5(0.0-1.0) [a]	1.0(0.25-1.75) [a]	32.5(26.25-38.75) [a]
Positive control	1.0(0.25-1.75) [b]	2.0(2.0-2.75) [c]	2.5(2.0-3.0) [b]	72.5(70.0-82.5) [c]
Nanoliposomes	1.5(1.0-2.0) [b]	1.0(1.0-1.75) [abc]	1.0(1.0-1.75) [a]	40.0(36.25-43.75) [a]
Eucerin	1.5(1.0-2.0) [b]	1.0(0.25-1.0) [ab]	1.0(1.0-1.75) [a]	35.0(31.25-38.75) [a]
Extract	1.0(1.0-1.0) [b]	2.0(1.25-2.0) [bc]	2.0(2.0-2.75) [ab]	62.5(56.25-68.75) [b]
Treatment	0.0(0.0-0.0)[a]	4.0(4.0-4.0) [d]	4(4.0-4.0) [c]	100.0(96.25-100.0) [d]

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

Two by two compared with the Mann-Whitney method showed that on 10th day, the amount of collagen deposition between the treatment group and negative control group (p = 0.001), nanocarrier control (p = 0.004), and Eucerin (p = 0.001) was statistically significant at the level (p <0.01). There was also a significant difference between the treatment group and the positive control group (p = 0.015) and extract (p = 0.017) in the amount of collagen deposition. The results of the Kruskal-Wallis test showed that on 10th day, there was a significant difference in the amount of re-epithelialization between the studied groups ($\chi 2$ (5) = 19.597, p = 0.001). Two by two comparison with Mann-Whitney method showed on 10th day in the amount of re-epithelialization between the treatment group with the negative group control (p = 0.001), nanocarrier control (p = 0.005), and Eucerin (p = 0.002). There was also a significant difference in the amount of re-epithelialization between the treatment group and the positive control group (p = 0.017) and extract (p = 0.019). The results of the Kruskal-Wallis test showed that on 10th day, there was a significant difference in the amount of neovascularization between the studied groups ($\chi 2$ (5) = 18.012, p = 0.003). Two by two compared with the Mann-Whitney method showed that on 10th day, the amount of neovascularization between the treatment group and negative control group (p = 0.001), nanocarrier control (p = 0.002), and Eucerin (p = 0.002) was statistically significant. There was also a significant difference in neovascularization between the treatment group and the positive control group (p = 0.015) and extract (p = 0.017).

The results of the Kruskal-Wallis test showed that on 15th day (Table 5), there was a significant difference in the amount of acute inflammation between the studied groups ($\chi 2$ (5) = 13.645, p = 0.018). Two by two compared with the Mann-Whitney method showed that on 15th day, there was a statistically significant difference in the amount of acute inflammation between the treatment group and the negative control group (p = 0.001), nanocarrier control (p = 0.006), and Eucerin (p = 0.006). There was also a significant

difference in acute inflammation between the treatment group and the positive control group (p = 0.046) and (p = 0.008) extract. Two by two compared with the Mann-Whitney method showed a statistically significant difference between the treatment group and the negative control group (p = 0.001), nanocarrier control (p = 0.009), and Eucerin (p = 0.001) on 15th day in the amount of granulation tissue maturation. There was also a significant difference in granulation tissue maturation between the treatment group and the positive control group (p = 0.011) and extract (p = 0.011). The results of the Kruskal-Wallis test showed that on 15th day, there was a significant difference in the amount of collagen deposition between the studied groups ($\chi 2$ (5) = 18.002, p = 0.003). Two by two comparison with the Mann-Whitney method showed that on 15th day, the amount of collagen deposition between the treatment group and negative control group (p = 0.001), nanocarrier control (p = 0.002), and Eucerin (p = 0.002) were statistically significant. There was also a significant difference in the amount of collagen deposition between the treatment group and the zinc oxide group (p = 0.013) and extract (p = 0.011). The results of the Kruskal-Wallis test showed that on 15th day, there was a significant difference in the amount of re-epithelialization between the studied groups ($\chi 2$ (5) = 20.830, p = 0.001). Two by two comparison with Mann-Whitney method showed on 15th day in the amount of re-epithelialization between the treatment group with the group negative control (p = 0.001), nanocarrier control (p = 0.007), and Eucerin (p = 0.001) statistically significant. There was also a significant difference between the treatment group and the positive control group (p = 0.017) and extract (p = 0.018) in the amount of re-epithelialization.

Histopathological Evaluation

Healing processes in burns in different groups on the 5th day, composed of the negative control group (a), extract group (b), treatment group (c), positive control group (d), control group of nanoliposomes (e), Eucerin control group (f) is shown in Figure 7. The negative control, nanoliposomes, and Eucerin groups had similar histopathological characteristics. They had the highest necrotic tissue and scab on the burn site (Figures 7a, e, and f). They showed minimal re-epithelialization and granulation tissue formation. Treatment group showed the best progress of the healing process among different groups containing the highest of re-epithelialization and granulation tissue formation and the lowest amount of necrotic tissue (Figure 7c). Extract group and positive control group showed similar healing process and better than the control, nano, and Eucerin groups and worse than treatment group (Figures 7b and d).

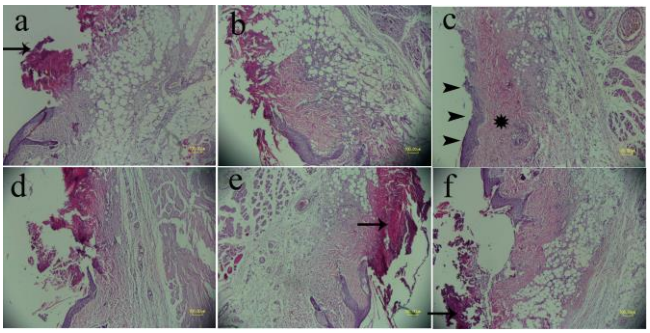

Figure 7. This photomicrograph shows healing processes in burns in different groups on the 5th days. The negative control group (a), control group of nanoliposomes (e), Eucerin control group (f) show high amount of necrotic tissue (arrows) on the burn site and have minimal re-epithelialization and granulation tissue formation. In the treatment group (c) the highest of re-epithelialization (arrowheads) and granulation tissue formation (asterisk) and the lowest amount of necrotic tissue are seen. In extract group (b) and positive control group (d) the healing process are better than the negative control, nanoliposomes and Eucerin groups and worse than treatment group. H&E.

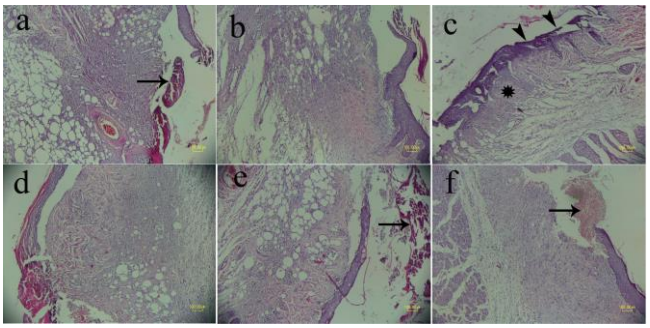

Figure 8. This photomicrograph shows healing processes in burns in different groups on the 10th days. In the negative control group (a), control group of nanoliposomes (e) and Eucerin control group (f), low amount of re-epithelialization, granulation tissue formation and collagen deposition are seen. Also, necrotic tissue and scab on the burn are present (arrows). In treatment group (c), partial re-epithelialization (arrow heads) and large amount of granulation tissue formation and collagen deposition (asterisk) are seen. Photomicrograph of extract group (b) and positive control group (d) show re-epithelialization, granulation tissue formation and collagen deposition between the negative, nanoliposomes and Eucerin control groups and treatment groups. H&E.

Photomicrograph in Figure 8 showed healing processes in burns in different groups on the 10th day, composed of the negative control group (a), extract group (b), treatment group (c), positive control group (d), control group of nanoliposomes (e), Eucerin control group (f).

In negative control, nanoliposomes, and Eucerin groups, the healing process included re-epithelialization, granulation tissue formation, and collagen deposition were slower than in the other groups (Figures 8a, e, and f). In the treatment group, the best healing process among all groups containing partial re-epithelialization, granulation tissue formation, and collagen deposition was seen (Figure 8c). Photomicrograph of extract group and positive control group reveal re-epithelialization, granulation tissue formation, and collagen deposition between the control, nano, and Eucerin groups and treatment group (Figures 8b and d).

The photomicrograph of Figure 9 showed healing processes in burns in different groups on the 15th day, composed of the negative control group (a), extract group (b), treatment group (c), positive control group (d), control group of nanoliposomes (e), Eucerin control group (f). In the negative control, nanoliposomes, and Eucerin groups, the re-epithelialization was significantly more incomplete than other groups. The scab adhered to the burn site. Many granulation tissues with high cellularity and angiogenesis related to the proliferative phase of healing were seen (Figures 9a, e, and f). Although the extract group and positive control group were in the proliferative phase of healing, the amount of re-epithelialization and maturity of granulation tissue was better than the control, nano, and Eucerin groups. Granulation tissue had less cellularity and angiogenesis and more collagen deposition (Figures 9b and d). In the treatment group, histopathological characteristics of the remodeling phase of the healing process were seen. Re-epithelialization was approximately completed. Granulation tissue was composed of a large amount of collagen and low cellularity and angiogenesis. The progress of the healing process in this group was better than in other groups (Figure 9c).

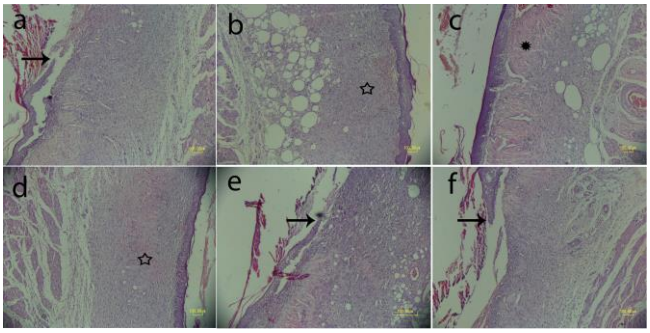

Figure 9. This photomicrograph shows healing processes in burns in different groups on the 15th days. In the negative control group (a), control group of nanoliposomes (e) and Eucerin control group (f), the re-epithelialization is incomplete (arrows). Scab is adhered on the burn site. Large amount of granulation tissue with high cellularity and angiogenesis related to proliferative phase of healing are seen. In treatment group (c) re-epithelialization is completed. Granulation tissue is mature and composed of large amount of collagen and low cellularity and angiogenesis (asterisk).in the extract group (b) and positive control group (d) re-epithelialization is approximately completed. Granulation tissue has low cellularity and angiogenesis and high collagen deposition (hollow stars) but maturity of granulation tissue is between the negative control, nanoliposomes and Eucerin groups and treatment group. H&E.

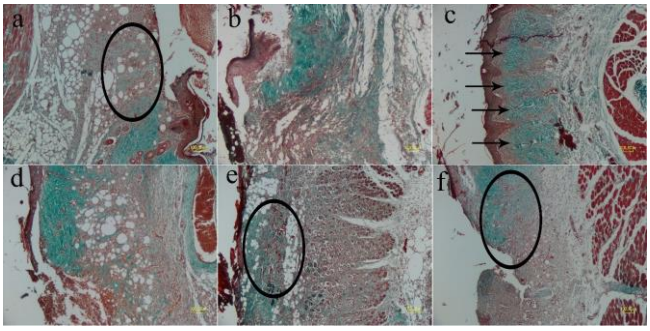

Figure 10. This photomicrograph shows collagen deposition as green color in burns in different groups on the 10th days. In the negative control group (a), control group of nanoliposomes (e) and Eucerin control group (f), low amount of deposition are seen (in the hollow oval). In treatment group (c), large amount of collagen deposition in the dermis along of the burned area (arrows) are seen. Photomicrograph of extract group (b) and positive control group (d) show collagen deposition between the negative control, nanoliposomes and Eucerin groups and treatment group. Masson's trichrome.

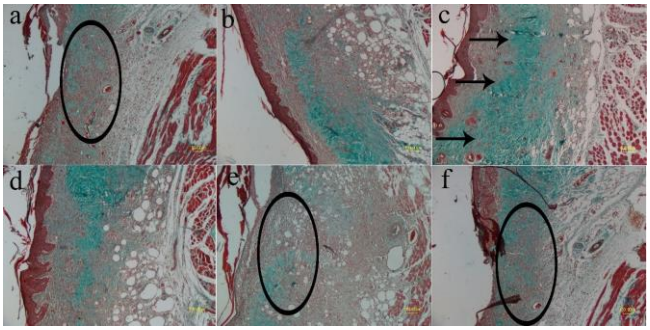

Figure 11. This photomicrograph shows collagen deposition as green color in burns in different groups on the 15th days. In the negative control group (a), control group of nanoliposomes (e) and Eucerin control group (f), collagen deposition is low, specially, in the center of burned area (in the hollow oval). In treatment group (c) large amount of thick collagen bundles in the dermis along of the burned area (arrows) are seen. In the extract group (b) and positive control group (d) the amount of collagen deposition is between the negative control, nanoliposomes and Eucerin groups and treatment group. Masson's trichrome.

Discussion

Wound healing is a complex, dynamic, and systematic process involving the interaction of multiple growth factors, cells, and matrix molecules that can be divided into four phases that overlap in time and space, including a hemostasis phase, inflammatory phase, proliferative phase, and remodeling phase[24]. Scrophularia is a well-known plant in Iranian traditional medicine. Its anti-oxidative and anti-inflammatory properties make it a logical adjuvant to improve wound healing. Ghashghaii *et al.* evaluated the potential of *Scrophularia striata* on wound healing of rats. During the experiment, treated rats with *Scrophularia striata* showed a significant decrease in the wound area compared to other groups. Additionally, treatment with *Scrophularia striata* decreased the number of lymphocytes. It enhanced the number of fibroblasts at the earlier stages. It increased the number of fibrocytes at the later stages of wound healing. Other parameters such as alignment of the healing tissue, re-epithelialization and epithelial formation, enhanced maturity of the collagen fibers and fibroblasts, and large capillary-sized blood vessels showed significant changes compared to control. The best wound healing activity was observed with the high dose of *Scrophularia striata.*[7] The same results were also reported by Stevenson *et al*. that compounds of *Scrophularia nodosa* were shown in vitro to stimulate the growth of human dermal fibroblasts.[25] In the present study, fibroblasts were enhanced, and it was consistent with Stevenson *et al*. results.[25] On the other hand, the number of fibrocytes increased at the later stages of wound healing. Over recent decades, much research effort has been invested in developing phytochemicals as wound healing agents. However, several impediments to their widespread use as drugs still have to be overcome. These limitations are low solubility, poor penetration into skin, high hepatic disposition, and narrow therapeutic index. Rapid clearance or uptake by normal tissues and wide tissue distribution results in low drug accumulation in the target sites can result in undesired drug exposure in normal tissues. Encapsulation in nanoscale drug carriers is a potential strategy to address these problems.[26]

In the last few decades, researchers have used herbal nanoliposome to treat and manage a variety of diseases like wound healing. Presently, several liposomal formulations are on the market for the treatment of diseases.[27] In this light, many researchers focused on the encapsulation of phytochemicals in liposomes to increase their bioavailability and wound healing effectiveness. For example, Takahashi *et al.* showed liposomes encapsulating aloe vera leaf gel extract significantly enhance proliferation and collagen synthesis in human skin cell lines. Hence, aloe vera leaf gel should have great potential as an effective skincare formulation.[28] The present study showed that *Scrophularia striata* can significantly decrease the wound area and, as aloe vera leaf gel, it can help strengthen the wounded skin. In another study, Cui *et al*. investigated the therapeutic effects of Danggui Buxue extract-loaded liposomes in thermosensitive gel on dorsal full-thickness excisional wounds in rats by measuring the percentage of wound contraction and hydroxyproline content, as well as conducting histological observations and immunohistochemical analysis.[24] The results show that Danggui Buxue extract-loaded liposomes in thermosensitive gel treatment remarkably accelerates wound closure, enhances hydroxyproline content in wound granulation tissue, promotes cutaneous wound healing by reducing the inflammatory response and improving fresh granulation tissue formation. It significantly increases the density of blood vessels, cell proliferation, and expression of type I and type III collagen. Moreover, Danggui Buxue extract-loaded liposomes in thermosensitive gel markedly upregulates the relative protein expression of vascular endothelial growth factor and transforming growth factor beta 1 and notably stimulates the phosphorylation of protein kinase B and small mothers against decapentaplegic 2/3. Their histological results showed that Danggui Buxue extract-loaded liposomes in thermosensitive gel accelerates full-thickness excisional wound healing, similar to our *Scrophularia striata* treated rats which had a significant decrease in the wound area. They suggested significant improvements in inflammatory cell infiltration, macrophage and fibroblast proliferation, angiogenesis, collagen synthesis, and re-epithelialization, forming a dense and uniform neotissue structure. In the present study, we showed that alignment of the healing tissue, re-epithelialization and epithelial formation, enhanced maturity of the collagen fibers and fibroblasts, and large capillary-sized blood vessels showed significant changes. It was pretty consistent with Cui *et al.* study.

Wu *et al*., in a comprehensive study, reported that liposomal farnesol (a natural 15-carbon organic

compound) from 0.04 mM to 0.8 mM significantly enhanced collagen production by murine skin fibroblasts.[29] In contrast, liposomal farnesol at high (0.8 mM) and low concentration (0.04 mM) did not show any suppressions on skin fibroblast proliferation. They treated third-degree burns on a rat model with a formulated gel composed of various ratios of 2% hydroxypropyl methylcellulose (HPMC) and 4 mM liposomal farnesol for 7 and 14 days. On days 7 and 14 post wounding, histopathological observations revealed that the HPMC: farnesol gel ratios of 1:2 and 2:1 exerted the most excellent tissue-repairing effects on the skin after third-degree burns compared with skin untreated or treated with a commercial burn gel and HPMC alone. These findings were consistent with the in vivo quantitative collagen-producing assay, wound healing scoring, and IL-6 Western blot results. They reported that liposomal farnesol, based on the concentration, had different results. The suppressions on skin fibroblast proliferation may differ significantly. The present study showed that the best wound healing activity was observed with the high dose of *Scrophularia striata* on different days. Treatment group showed the best progress of the healing process among other groups containing the highest of re-epithelialization and granulation tissue formation and the lowest amount of necrotic tissue.

Scrophularia is a promising wound dressing for dermal wound healing. Further, loading Scrophularia extracts into topical sustained-release drug delivery systems is a novel and beneficial strategy for improving cutaneous wound healing. Liposomes tend to fuse to the cell's phospholipid bilayer membrane. Liposomes are less flexible than membranes, which slows drug release and reduces drug toxicity and side effects. There are many benefits to using liposomes on the skin. Liposomes are safe and biodegradable materials. They have unparalleled compatibility and affinity for skin that can improve the permeability of the stratum corneum and penetrate the skin rapidly. Liposomes can be applied directly to skin lesions. Liposomes have shown antioxidant and wound healing properties. It protects the plant extract against proteases that are at the wound site. This substance protects the plant extract against proteases that are at the wound site. Nanoliposomes increase intracytoplasmic permeability relative to the non-nano particle state and also increase the drug concentration at the site.

In summary, the obtained results showed that the size of the treatment group was smaller than other groups and had a better effect on repairing wound healing. The positive control had a better impact on wound repair than extract, Eucerin, nanoliposomes, and negative groups. According to the highest re-epithelialization and granulation tissue formation and the lowest necrotic tissue, we can suggest that the treatment group has the best healing process among different groups.

In conclusion, *Scrophularia striata* extract loaded-liposome has a nanometric range, spherical morphology and good effect on the healing of burn wounds in rats. It is suggested to evaluate the effect of other herbs in a combination of liposome nanocarrier and *Scrophularia striata* extract with different nanocarriers on burn wound healing.

Conflict of Interest

The authors declare no conflict of interest.

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