Iranian Veterinary Surgery Association

Iranian Journal of Veterinary Surgery

Journal homepage: www.ivsajournals.com

Original Article

Hydrogel Based on Alginate-Polyethylene Glycol Polymer Containing *Scrophularia striata* Extract Nano-Liposomes is an Excellent Nano-Phyto-Extract in Wound Healing; a Geometric and Pathological Study

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ARTICLE INFO

Article History:

Received 23 September 2022
Revised 7 November 2022
Accepted 14 December 2022
Online 14 December 2022

Keywords:

Scrophularia striata
Wound healing
Hydrogel
Alginate
Polyethylene glycol

ABSTRACT

Due to the anti-inflammatory and antioxidant properties of *Scrophularia striata*, it is used to heal wounds. Today, hydrogel based on alginate-polyethylene glycol polymer is mainly used due to its drug delivery properties and create of suitable conditions for wound healing. In this study, we tried to investigate the effect of hydrogel based on alginate-polyethylene glycol polymer containing *Scrophularia striata* extract nanoliposomes on wound healing in rat animal models. Field emission scanning electron microscopy and dynamic light scattering were used to examine nanoliposome and hydrogel. 126 Wistar albino rats were randomly divided into 7 groups (18 rats/group). The rats were anesthetized and their dorsum shaved, a burn wound was created with a cylindrical copper at 100° C. Rats' wounds were treated with hydrogel *Scrophularia striata* extract and on the 5th, 10th, and 15th days, histopathological evaluation, macroscopic features and wound healing were evaluated and analyzed in different groups. The structure of nanoliposomes was uniform and the size was 80-110 nm. Also, the size of the hydrogel was 320 nm with nanometric size and spherical morphology. Histopathological evaluation, wound area and wound contraction confirmed that the treatment group had a significant difference from other groups and the effect was almost similar to that of zinc oxide. This study showed that alginate-polyethylene glycol polymer containing *Scrophularia striata* extract caused wound contraction, and reduced wound area, and can be used for wound healing.

Introduction

The skin of the body is the first and most important defense barrier against infections and microorganisms.[1] In addition to protection, the skin plays a role in regulating water and electrolytes in the body and is also considered a metabolic and sensory organ.[1,2] Injuries and factors that cause the loss of skin integrity, damage

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https://doi.org/10.30500/ivsa.2022.363343.1322

to the dermis and epidermis are known as wounds.[3] Fire, high heat and caustic chemicals that cause damage to the skin are known as burns.[4,5] According to the reports of the World Health Organization, more than 180,000 deaths occur due to burns worldwide every year and it is considered one of the injuries that lead to death.[6,7] The main cause of death in burn wounds is the growth of microorganism's resistant to antibiotics and the production of biofilm. These microorganisms lead to the failure of antibiotic treatment and the spread of infection.[8,9] Also, antibiotic-resistant microorganisms may increase due to the insufficient concentration of local antibiotics used in the treatment of burn wounds.[10-12] In the absence of proper treatment and repair of the skin, the infection may spread in the body, Also the skin and finally the underlying tissues of the skin will be destroyed.[13,14] Antibiotics and chemicals may cause harmful side effects, so today the use of natural and herbal substances is expanding mainly in the pharmaceutical and medical industry.[15,16] *Scrophularia* is one of the most important plants used in Iranian traditional medicine to treat infectious and inflammatory diseases. The most important member of this family *is Scrophularia striata* and it is known by different names in Iran such as Ashineh, Teshneh Dari, Benj Ghan, Benjek and it is believed that it has a therapeutic effect on wound infections and burns.[17-19] The extract of this plant reduces edema, cellular infiltration, and the proliferation of T lymphocytes in the tissue, stimulates the production of collagen, fibroblast cells, and angiogenesis and also antibacterial effects.[20,21] Today, with the development of nanotechnology, nanoparticles are widely used in drug delivery. Nanoparticles such as liposomes are considered as a drug delivery system due to stable and controlled release, drug protection, passing through the biological barriers of cells to deliver the drug to the target site, biocompatibility, increasing the drug's permeability in the bloodstream and increase the efficiency of drugs.[22-24] Hydrogels form a three-dimensional structure by absorbing water and body fluids and allow hydrophilic polymers to form a network with each other. Due to having a large amount of water and permeability, this composition has the same function as living tissue.[25,26] Based on the origin of the polymer, hydrogels are classified into two groups: natural (alginate) and synthetic (polyethylene glycol [PEG]). Alginate is considered a wound dressing due to the absorption of water and body fluids as well as its elastic property that provides the necessary moisture for wound healing.[27,28] Also, due to the biocompatibility and hydrophilicity of PEG, this compound is used as a hydrogel in drug delivery.[29] Nanoparticles, especially alginate, have an antibacterial effect on Gram-positive and Gram-negative bacterial wounds. This characteristic has made these particles to be used as antibacterial agents alone or in combination with other compounds.[30]

Therefore, the purpose of this study was to investigate the effect of hydrogel based on alginate-polyethylene glycol polymer containing *Scrophularia striata* extract nano-liposomes on wound healing in rat animal models.

Materials and Methods

Preparation of Extract of Scrophularia striata

The *Scrophularia striata* plant was purchased from a local store. After being powdered, 75 g of it was placed in a Soxhlet and its compounds were extracted with 300 ml of ethanol (50%) by heating at 90° C for 10 h. After evaporation of the solvent by rotary, the product was collected and stored in a refrigerator at 5° C for later use.[17]

Synthesis of Scrophularia striata Liposomes

First, 1.0 g of lecithin was added to 50 ml of deionized water and stirred to dissolve. After the addition of plant extract to the above solution, this solution was sonicated for 30 min. Then it was mixed with a homogenizer. In this stage, the nano-liposome of *Scrophularia striata* was prepared and stored in the refrigerator at 10° C.[31]

Preparation of Liposomal Hydrogel

To prepare the liposomal hydrogel; 10 ml of the nanoliposome was added to 10 g of water (solution a). Polyethylene glycol and alginate were dissolved in 10 ml of water (solution B). The solution b was added to solution A and stirred vigorously for 30 min. The obtained liposomal hydrogel was stored in the refrigerator at 10 °C.[32]

Experimental Animals

In this study, 126 Wistar albino male rats with an average age of 2 to 3 months and an average weight of 200 to 300 g were prepared (animal laboratory of Kerman University of Medical Sciences, Kerman, Iran). The rats were randomly divided into 7 groups (positive control group (zinc oxide ointment), negative control

group (normal saline based on alginate-PEG polymer), treatment (hydrogel containing nanoliposomes loaded with extract based on alginate-PEG polymer), Hydrogel (alginate-PEG polymer hydrogel without plant extract), Nano-liposome (nanoliposome containing plant), Nano-extract and extract) including 18 rats and kept in optimal conditions of temperature (21 ± 1° C) light (12 h/12 h dark-light,) water and food. The rats were anesthetized intramuscularly using ketamine and xylazine (50 and 5 mg/kg, respectively) and the dorsal surface area according to the principles of surgical asepsis was shaved.[33,34] A cylindrical copper rod with a temperature of 100° C was used to cause burns (10 seconds without any pressure). The prepared hydrogel was applied topically on the burn wounds for 15 days (every 24 hours) and covered with a sterile dressing. For histopathological evaluation, on the 5th, 10th and 15th day, six mice were euthanized, skin samples were prepared and preserved in formalin.[35] The experimental protocols were conducted by the ethics committee of the Kerman University of Medical Sciences guidelines.

Geometric Evaluation

To check the healing process of the burn wound, daily photography (10 cm distance from the wound and using the L-shaped ruler) of the wound was done using a digital camera (Canon 1DS, Japan) on the days 0, 3rd, 6th, 9th , 12th and 15th, and then the healing process and the size of the wound were analyzed with Image j software(A Java imaging program free download from the internet).[36]

Histopathologic Evaluation

After fixing the samples, the samples were dehydrated using alcohol. After removing water, the samples were cleared in xylene, and embedded in paraffin wax. Sections in 5 µm thickness were prepared from each sample and after staining with hematoxylin-eosin and Masson's trichrome, the samples were examined with a light microscope. The photomicrographs prepared from the samples were examined to determine inflammation (infiltration of neutrophils, edema and hyperemia), re-epithelialization, granulation tissue formation, collagen deposition, and scar maturation.[35,37]

Statistical Analysis

The results of the geometry study were analyzed by SPSS statistical software and the One-way analysis of variance (ANOVA) statistical method and the results of histopathological studies were analyzed by the nonparametric method. In all stages of the analysis, the allowable error for rejecting the null hypothesis was considered 5%.

Results

Characterization of Nano-Metric Liposomes

Field emission scanning electron microscopy (FE-SEM) was used to investigate the surface morphology of the synthesized nanostructure. Figure 1 shows the FE-SEM image of the synthesized nanoliposome hydrogel. As can be seen from the figure, the nanoliposome structure is almost uniformly dispersed throughout the polymer substrate.

Also, the image confirms the size of nanoliposomes is between 80-110 nm. Also, to confirm the synthesized nanoparticles, the Dynamic light scattering (DLS) technique was used. Thus, the size distribution of the prepared nano-liposome hydrogel was obtained. As shown in Figure. 2, the mean diameter of nano-liposome was about 320 nm which was consistent with the results of the FE-SEM.

Comparison of the Average Wound Area in Rats

Figure 3 shows the changes in the wound area of rats on days 0, 3, 6, 9, 12 and 15 according to the compounds used. Based on this figure, at the end of the 15th day, the wound of the zinc oxide ointment group was completely healed and the average area of the wound was less than that of the treatment group (0.0 ± 0.0 mm^2). In rats treated with hydrogel containing nanoliposomes loaded with *Scrophularia striata* extract based on alginate-PEG polymer, complete healing was

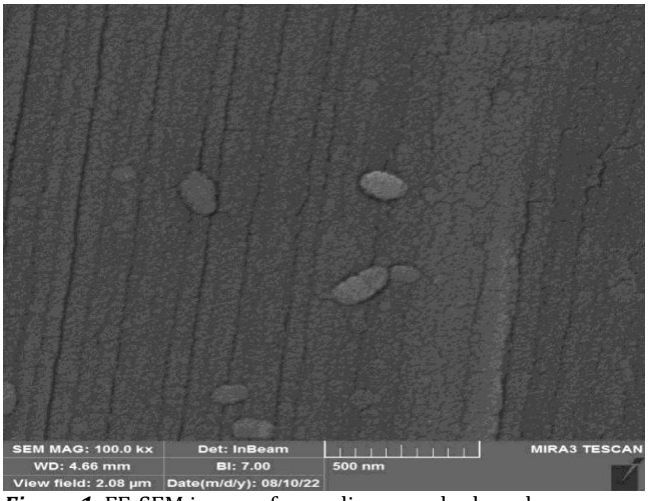

Figure 1. FE-SEM image of nano-liposome hydrogel.

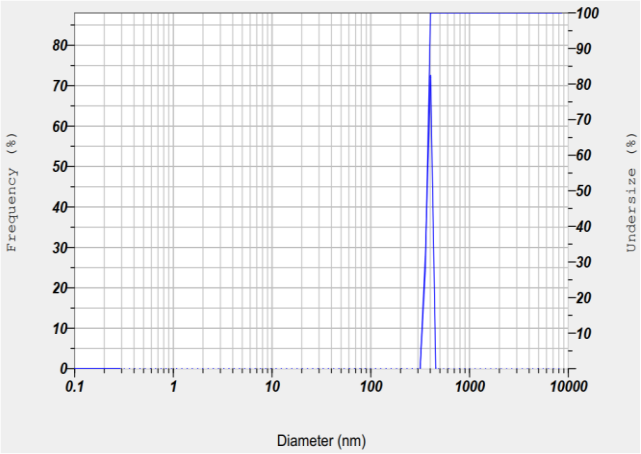

Figure 2. DLS of nano-liposome hydrogel.

not observed in only two rats' wounds and the average wound area was (0.0003 ± 0.0002 mm²). The highest value for the negative control group (normal saline based on alginate-PEG polymer) (0.218 ± 0.02 mm²).

One-way ANOVA and Tukey's supplementary test were performed to investigate the difference in the average wound area in rats during the healing period between the studied compounds and the results are presented in Table 1. Based on the results of ANOVA, between treatment, zinc oxide ointment, nano-liposome control, extract, nano-extract, hydrogel and negative control on the average wound area during the 6th day (F[6.77] = 13.510 and p = 0.001), 9th day (F[6.75] = 475.52 and p = 0.0001), 12th day (F[6.34] = 435.55 and p = 0.0001) and 15th day (F[6.34] = 45.033 and p = 0.0001) a significant difference was observed (p < 0.01). However, there was no significant difference in the average wound area between the studied groups on the first and 3th days (p > 0.05). The results of Tukey's supplemental test (Figure 4) showed that the average wound area in the rats of the treatment group and zinc oxide ointment on the sixth day was significantly less than the control groups of nanoliposome, extract, nano-extract, hydrogel and negative control (p < 0.05). However, no significant difference was observed between the control group of nanoliposome, extract, nano-extract, hydrogel and negative control. On the 9th day, the average area of the wound in the rats of the treatment group and zinc oxide ointment was significantly less than the control groups of nanoliposome, extract, nano-extract, hydrogel and negative control. Also, the average wound area of control group and nanoliposome is significantly higher than extract, nano-extract and hydrogel. (p < 0.05). On the 12th and 15th days, the same as the 9th day, the average area of the wound in the rats of the treatment group and zinc oxide ointment was significantly less than the control groups of nanoliposome, extract, nano-extract, hydrogel and negative control, and the average area of the wound in the rats of the control and nanoliposome groups were more significant than hydrogel. (p < 0.05).

Comparison of the Average Percentage of Wound Contraction in Rats

The results of changes in the percentage of wound contraction of rats according to the compounds studied and measured during days 0, 3, 6, 9, 12 and 15 were shown in Figure 5. According to this figure, the wound in the rats receiving zinc oxide ointment was completely healed at the end of the 15th day and the average percentage of wound contraction was higher than the treatment group (100.0% ± 0. 0%).In the rats of the treatment group, the average percentage of wound contraction was (99.96% ± 0.02%) and the wounds of the two rats were not completely healed. Also, the lowest percentage of wound contraction was in the negative control group (75.88% ± 1.70%).

One-way ANOVA test and Tukey's supplementary test were used to investigate the difference in the average percentage of wound contraction of rats during the healing period and the studied compounds (Table 2). In the examination of the samples of negative control, zinc oxide, treatment, hydrogel, nano liposome, nano-extract and extract on the average percentage of wound contraction during the third days (F[6.119] = 37.717 and p = 0.001), the sixth days (p = 0.001 and F[6.77] =69.860), the ninth day (p = 0.0001 and F[6.75] = 168.440), the twelfth day (p = 0.001 and F[6.34] = 97.236) and on the 15th day (p = 0.0001 and F[6.34] =

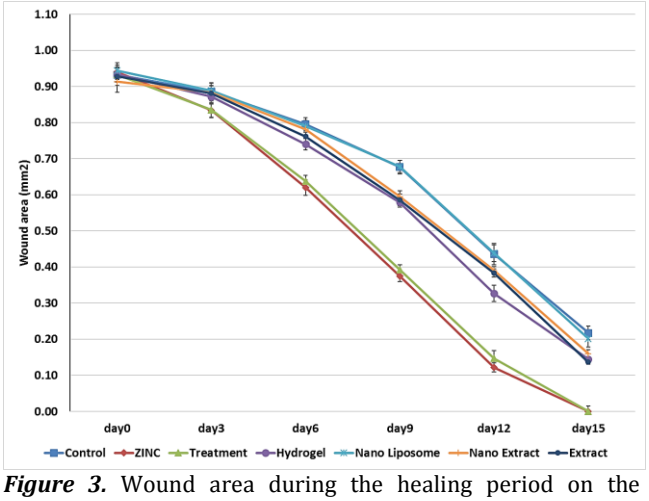

Figure 3. Wound area during the healing period on the treatment group on 0, 3rd ,6th, 9th, 12th, and 15th days after the initial operation.

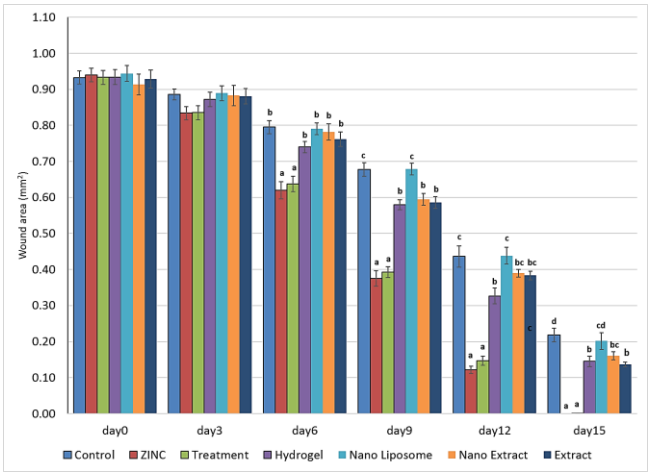

Figure 4. Comparison of the average wound area in rats according to the studied compounds with the Tukey method (95% probability level). The columns that have the same letters in each day have no significant difference.

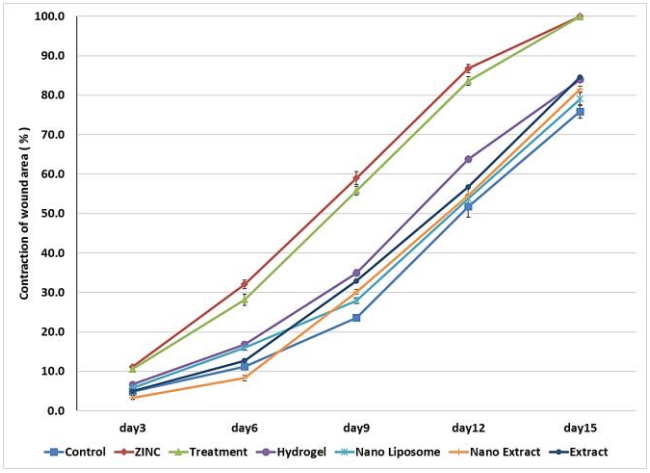

Figure 5. Wound average percentage (Mean ± SE) during the healing period on the treatment group on 0, 3rd ,6th, 9th, 12th, and 15th days after the initial operation.

Table 1. Effect of hydrogel based on polymer alginate-polyethylene glyco containing liposome nano-carrier *S. striata* extract on burn wound area (mm²) on various days of healing in Rats

Groups	**Wound area ± SD (mm²)**					
	0	**3**	**6**	**9**	**12**	**15**
Negative control	0.932 ± 0.07	0.885 ±0.06	0.795 ± 0.06 ^{b}	0.677 ± 0.06 ^{c}	0.436 ± 0.07 ^{c}	0.218 ± 0.04 ^{d}
Zinc oxide	0.939 ± 0.08	0.833 ± 0.08	0.620 ± 0.08 ^{a}	0.375 ± 0.07 ^{a}	0.121 ± 0.02 ^{a}	0.00 ± 0.00 ^{a}
treatment	0.932 ± 0.08	0.835 ± 0.08	0.637 ± 0.07 ^{a}	0.392 ± 0.05 ^{a}	0.146 ± 0.03 ^{a}	0.003 ± 0.00 ^{a}
Hydrogel	0.933 ± 0.09	0.871 ± 0.08	0.740 ± 0.05 ^{b}	0.579 ± 0.05 ^{b}	0.326 ± 0.05 ^{b}	0.145 ± 0.04 ^{b}
Nano-liposome	0.943 ± 0.09	0.888 ± 0.09	0.790 ± 0.05 ^{b}	0.678 ± 0.06 ^{c}	0.438 ± 0.06 ^{c}	0.201 ± 0.06 ^{cd}
Nano-extract	0.913 ± 0.12	0.882 ± 0.12	0.781 ± 0.08 ^{b}	0.595 ± 0.06 ^{b}	0.390 ± 0.03 ^{bc}	0.160 ± 0.03 ^{bc}
Extract	0.928 ± 0.10	0.881 ± 0.09	0.761 ± 0.07 ^{b}	0.585 ± 0.06 ^{b}	0.383 ± 0.03 ^{bc}	0.136 ± 0.01 ^{b}
Sig.	0.980	0.258	0.001	0.001	0.001	0.001
N in each group	18	18	12	12	6	6

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

Table 2. Effect of hydrogel based on polymer alginate-polyethylene glyco containing liposome nanocarrier *Scrophularia striata* extract on contraction of wound area (%) on various days of healing in Rats

Groups	**Contraction of wound area ± SD (%)**					
	0	**3**	**6**	**9**	**12**	**15**
Negative control	0.0 ± 0.0	4.93 ±2.43 ^{ab}	11.16 ± 1.97 ^{ab}	23.50 ± 2.23 ^{a}	51.70 ± 5.85 ^{a}	75.88 ± 3.81 ^{a}
Zinc oxide	0.0 ± 0.0	11.15 ± 1.50 ^{c}	32.05 ± 3.85 ^{f}	58.97 ± 5.66 ^{e}	86.74 ± 2.60 ^{c}	100.0 ± 0.00 ^{d}
treatment	0.0 ± 0.0	10.55 ± 1.58 ^{c}	28.15 ± 4.98 ^{e}	55.78 ± 3.72 ^{e}	83.74 ± 2.62 ^{c}	99.96 ± 0.05 ^{d}
Hydrogel	0.0 ± 0.0	6.68 ± 1.04 ^{b}	16.83 ± 2.15 ^{d}	34.94 ± 2.15 ^{d}	63.78 ± 4.56 ^{b}	83.96 ± 3.41 ^{c}
Nano-liposome	0.0 ± 0.0	5.80 ± 2.73 ^{b}	15.95 ± 5.97 ^{cd}	27.91 ± 4.96 ^{b}	53.76 ± 4.95 ^{a}	79.02 ± 3.96 ^{ab}
Nano-extract	0.0 ± 0.0	3.33 ± 2.17 ^{a}	8.26 ± 2.19 ^{a}	30.09 ± 1.95 ^{bc}	54.62 ± 0.67 ^{a}	81.47 ± 2.06 ^{bc}
Extract	0.0 ± 0.0	4.97 ± 2.34 ^{ab}	12.72 ± 2.56 ^{bc}	32.07 ± 2.90 ^{cd}	56.72 ± 1.37 ^{a}	84.59 ± 1.11 ^{c}
Sig.	1.0	0.001	0.001	0.001	0.001	0.001
N in each group	18	18	12	12	6	6

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

83.910) a significant difference was observed (p < 0.01).

On the third day, Tukey's supplemental test (Figure 6) showed that the average percentage of wound contraction in the rats of the treatment group and zinc oxide ointment was significantly higher than the control groups of nanoliposome, extract, nano-extract, hydrogel and negative control (p < 0.05). Also, no significant difference was observed between the control groups of nanoliposome, extract, hydrogel and negative control.

In general, on the 6th, 9th, 12th, and 15th days, the average percentage of wound contraction in the rats of the treatment group and zinc oxide ointment was significantly higher than the control groups of nanoliposome, extract, nano-extract, hydrogel, and negative control (p < 0.05). In the treatment group, the average percentage of wound contraction was significantly lower than that of zinc oxide ointment (p < 0.05). Also, the average percentage of wound contraction in the control group was significantly lower than that of nanoliposome and hydrogel. (p < 0.05).

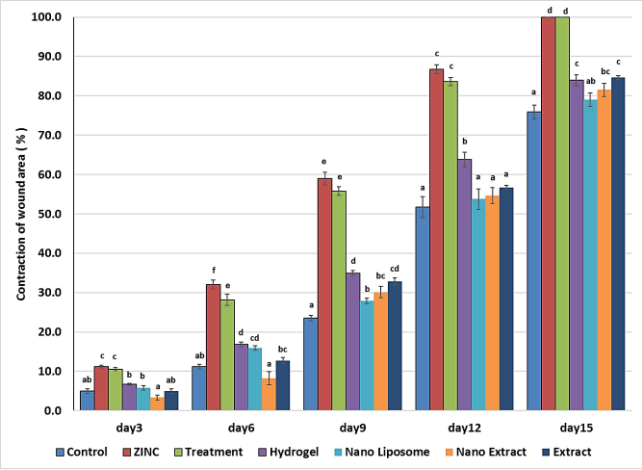

Figure 6. Comparison of the average percentage of wound contraction in rats according to the studied compounds with the Tukey's supplemental test (95% probability level). The columns that have the same letters in each day have no significant difference.

Histopathological Evaluation

The histopathological results on the 5th, 10th and 15th day are presented in Tables 3, 4, and 5. Table 3 shows the histopathologic changes of the groups on the 5th day after exposure to different treatments.

According to the Kruskal-Wallis test, on 5th day between the studied groups, there was no significant difference in the presence of neutrophils ($\chi2[6]$ = 8.370, p = 0.212), the amount of granulation tissue formation ($\chi2[6]$ = 7.556, p = 0.273) and vascularization ($\chi2[6]$ = 7.344, p = 0.290). Although the results of this test showed a significant difference in the amount of the inflammation phase of repair ($\chi2[6]$ = 18.739, p = 0.005) and necrotic tissue ($\chi2[5]$ = 18.524, p = 0.002).

Data analysis using the Mann-Whitney test in the amount of healing inflammation phase, showed that there is a significant difference (p < 0.01) between the group treated with liposome (p = 0.007), nano-extract (p = 0.007) and hydrogel (p = 0.007). Also, there was a significant difference in the level (p < 0.05) between the treatment group with negative control (p = 0.030) and extract (p = 0.030). However, there was no significant difference between the treatment and zinc oxide groups.

In the amount of necrotic tissue, there was a statistically significant difference (p < 0.01) between the treatment group with negative control (p = 0.003) and extract groups (p = 0.003). Although there was a significant difference (p < 0.05) between the treatment group, liposome (p = 0.011), nano-extract (p = 0.011) and hydrogel (p = 0.038), however, there was no significant relationship between the treatment group and zinc oxide.

According to the Kruskal-Wallis test, on the 10th day (Table 4) there is no significant difference between the studied groups in the amount of inflammation ($\chi2[6]$ = 7.556, p = 0.273) and collagen formation ($\chi2[6]$ = 10.997, p = 0.088) (p > 0.05), Although there is a significant difference between the studied groups in the amount of granulation tissue formation ($\chi2[6]$ = 26.310, p = 0.001) and the amount of vascularization ($\chi2[6]$ = 19.483, p = 0.003) .Mann-Whitney test shows a significant difference (p < 0.01) in the amount of granulation tissue formation between the treatment group and the negative control group (p = 0.004), liposome (p = 0.001), hydrogel (p = 0.004) and nano-extract (p = 0.001). Also, there was a significant difference between the treatment group and the extract group (p = 0.014) in the amount of granulation tissue formation, while there was no significant difference between the zinc oxide group. There is a significant difference in the amount of angiogenesis between the treatment group with nano-extract (p = 0.005), liposome (p = 0.001), hydrogel (p = 0.005), negative control (p = 0.020) and extract (p = 0.020) groups, however, no significant difference was observed between the treatment and zinc oxide groups.

The histopathological results on the 15th day between the studied groups are shown in Table 5.

According to Table 5, the results of the Kruskal Wallis test show a significant difference in the amount of granulation tissue formation ($\chi2[6] = 19.241$, $p = 0.004$), the arrangement of collagens ($\chi2[6] = 20.328$, $p = 0.002$) and the amount of vascularization ($\chi2[6] = 23.905$, $p = 0.001$) between the studied groups. A two-by-two comparison with the Mann-Whitney method showed that there is a statistically significant difference in the amount of granulation tissue formation between the group treated with liposome ($p = 0.004$) and nano-extract ($p = 0.004$) ($p < 0.01$). Also, there was a significant difference in the level ($p < 0.05$) between the group treated with hydrogel ($p = 0.015$) and extract ($p = 0.015$). However, there was no significant difference in the amount of granulation tissue formation between the treatment group and the negative control and zinc oxide groups. On 15th day, the amount of collagen arrangement between the treatment group with the negative control ($p = 0.004$), liposome ($p = 0.004$) and extract ($p = 0.004$) groups and also between the treatment group with the hydrogel ($p = 0.014$) and nano-extract ($p = 0.043$) groups there was a significant difference, but there is no significant difference between the treatment group and the zinc oxide group.

Also, according to Table 5, the amount of angiogenesis between the treatment group with the nano-extract ($p = 0.001$), liposome ($p = 0.005$) and extract ($p = 0.001$) groups and between the treatment group with the negative control ($p = 0.016$) and hydrogel ($p = 0.016$) groups was a significant difference ($p < 0.05$). However, there was no significant difference between the treatment and zinc oxide groups in the amount of angiogenesis.

Comparison of the Average Percentage of Epithelial Tissue Formation and Rearrangement

The mean ± standard deviation (SD) of the percentage of epithelial tissue formation and rearrangement in rats during the treatment period (5th, 10th and 15th day) between the studied compounds is shown in Table 6. The results of ANOVA and Tukey's and Tukey's supplementary test in evaluating the effect

Table 3. The effect of hydrogel based on polymer alginate-polyethylene glyco containing liposome nanocarrier containing *Scrophularia striata* extract on histological changes on day 5 of healing in rats. The data were expressed as median (25th percentile, 75th percentile).

Groups	Acute inflammation	Formation of granulation tissue	Inflammation phase	Neovascularization	Necrotic tissue
Negative control	1(0.5-1.5) [a]	1.0(1.0-1.0) [a]	1.0(1.0-2.0) [a]	1.0(1.0-1.5) [a]	4.0(3.0-4.0) [b]
Zinc oxide	0.0(0.0-0.5) [a]	1.0(1.0-2.0) [a]	2.0(2.0-3.0) [b]	2.0(1.0-2.0) [a]	2.0(2.0-3.0) [a]
Treatment	0.0(0.0-1.0) [a]	2.0(1.0-2.0) [a]	2.0(2.0-3.0) [b]	2.0(1.0-2.0) [a]	2.0(2.0-2.5) [a]
Hydrogel	1.0(0.5-1.5) [a]	1.0(1.0-1.5) [a]	1.0(1.0-1.5) [a]	1.0(1.0-1.5) [a]	3.0(3.0-3.5) [b]
Nano-liposome	1.0(0.5-1.0) [a]	1.0(1.0-1.5) [a]	1.0(1.0-1.5) [a]	1.0(1.0-1.0) [a]	3.0(3.0-4.0) [b]
Nano-extract	1.0(0.5-1.5) [a]	1.0(1.0-1.0) [a]	1.0(1.0-1.5) [a]	1.0(1.0-1.5) [a]	3.0(3.0-4.0) [b]
Extract	1.0(0.5-1.5) [a]	1.0(1.0-1.5) [a]	1.0(1.0-2.0) [a]	1(1.0-1.5) [a]	4(3.0-4.0) [b]
Sig.	0.212	0.273	0.005	0.290	0.005

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

Table 4. The effect of hydrogel based on polymer alginate-polyethylene glyco containing liposome nanocarrier containing *Scrophularia striata* extract on histological changes on 10th day of healing in rats.

Groups	Acute inflammation	Formation of granulation tissue	Collagen deposition	Neovascularization
Negative control	2.0(1.5-2.0) [a]	2.0(1.5-2.0) [a]	2.0(1.5-2.5) [a]	3.0(2.0-3.0) [a]
Zinc oxide	1.0(1.0-1.5) [a]	3.0(3.0-3.5) [b]	3.0(2.5-3.5) [a]	4.0(3.0-4.0) [b]
Treatment	1.0(1.0-1.5) [a]	3.0(3.0-3.5) [b]	3.0(2.5-4.0) [a]	4.0(3.5-4.0) [a]
Hydrogel	1.0(1.0-2.0) [a]	2.0(1.5-2.0) [a]	2.0(2.0-2.5) [a]	2.0(2.0-3.0) [ab]
Nano-liposome	2.0(1.5-2.0) [a]	2.0(1.0-2.0) [a]	2.0(2.0-3.0) [a]	2.0(2.0-2.5) [ab]
Nano-extract	1.0(1.0-2.0) [a]	2.0(1.0-2.0) [a]	2.0(2.0-2.5) [a]	2.0(2.0-3.0) [ab]
Extract	2.0(1.0-2.0) [a]	2.0(2.0-2.0) [a]	2.0(2.0-3.0) [a]	3.0(2.0-3.0) [c]
Sig.	0.273	0.001	0.088	0.003

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

Table 5. The effect of hydrogel based on polymer alginate-polyethylene glyco containing liposome nanocarrier containing *S. striata* extract on histological changes on day 15 of healing in rats.

Groups	Formation of granulation tissue	Collagen deposition	Neovascularization
Negative control	3.0(2.5-3.5) ^{ab}	1.0(1.0-2.0) ^{a}	3.0(2.5-3.0) ^{a}
Zinc oxide	4.0(3.5-4.0) ^{b}	3.0(2.5-3.5) ^{b}	4.0(3.5-4.0) ^{c}
Treatment	4.0(3.5-4.0) ^{b}	3.0(2.5-4.0) ^{a}	4.0(4.0-4.0) ^{a}
Hydrogel	3.0(2.0-3.0) ^{a}	2.0(1.0-2.0) ^{a}	3.0(2.5-3.0) ^{a}
Nano-liposome	2.0(2.0-3.0) ^{a}	1.0(1.0-2.0) ^{a}	3.0(2.0-3.0) ^{a}
Nano-extract	2.0(2.0-3.0) ^{a}	2.0(1.5-2.0) ^{ab}	2.0(2.0-2.5) ^{b}
Extract	3.0(2.0-3.0) ^{a}	1.0(1.0-2.0) ^{c}	2.0(2.0-3.0) ^{d}
Sig.	0.004	0.002	0.001

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

Table 6. The effect of hydrogel based on polymer alginate-polyethylene glyco containing liposome nanocarrier containing *Scrophularia striata* extract on epithelial tissue rearrangement in 5th, 10th and 15th day of healing in rats. The data were expressed as mean ± SD.

Groups	Day		
	5	10	15
Negative control	12.00 ± 4.47 ^{a}	35.00 ± 3.53 ^{a}	75.00 ± 3.53 ^{a}
Zinc oxide	26.00 ± 4.18 ^{b}	56.00 ± 4.18 ^{b}	96.00 ± 4.18 ^{b}
Treatment	28.00 ± 2.73 ^{b}	59.00 ± 5.47 ^{b}	97.00 ± 2.73 ^{b}
Hydrogel	12.00 ± 2.73 ^{a}	37.00 ± 4.47 ^{a}	75.00 ± 5.00 ^{a}
Nano-liposome	11.00 ± 2.23 ^{a}	37.00 ± 5.70 ^{a}	76.00 ± 4.18 ^{a}
Nano-extract	12.00 ± 2.73 ^{a}	35.00 ± 3.53 ^{a}	74.00 ± 4.18 ^{a}
Extract	11.00 ± 4.18 ^{a}	36.00 ± 4.18 ^{a}	74.00 ± 4.18 ^{a}
Sig.	0.001	0.001	0.001

N= 6 animals in each group. Data are presented as the mean ± SD. There are significant differences between groups with different codes (superscript letters a, b, c; $p < 0.05$ vs. carrier control).

of treatment, zinc oxide ointment, nanoliposome control, extract, nano-extract, hydrogel and negative control on the average percentage of epithelial tissue formation and rearrangement during the 5th day (F = 6.28 and $p = 0.001$), 10th day (F = 6.28 and $p = 0.0001$) and on the 15th day (F = 6.28 and $p = 0.0001$) showed a significant difference ($p < 0.01$). The results of Tukey's supplementary test showed that the average percentage of epithelial tissue formation on the 5th day and the average percentage of epithelial tissue rearrangement on the 10th and 15th day in the rates of the zinc oxide ointment and treatment group were significantly higher than the nanoliposome, extract, nano-extract, hydrogel and negative control groups ($p < 0.05$). While there was no significant difference between nanoliposome control, extract, nano-extract, hydrogel and negative control.

Also, burn healing processes in different groups on the 5th day are shown in Figure 7. according this figure, In the negative control group (a and b), hydrogel group (f), liposome group (g), nano-extract group (h), extract group (i) large amount of necrotic tissues are seen. The healing processes including re-epithelialization and granulation tissue formation are weak. In the treatment group re-epithelialization with high thickness are formed under the scab (c). Also, early stage of immature granulation tissue formation is seen (d). in the positive control group (e) the re-epithelialization and granulation tissue formation are similar to the treatment group.

The 10th day burn healing processes are shown in Figure 8. In the 10th day, the negative control group (a), shows low re-epithelialization in the margin of the burn site. Also, immature granulation tissue with low amount of collagen deposition beneath of the scab are seen (b). In the treatment group (c and d), high percent of the burn surface are covered with new epidermis. Large amount of granulation tissue with collagen synthesis in the deeper and more immature granulation tissue in the superficial part of the burn site are seen. In the positive control group (e), partial re- epithelialization and large amount of granulation tissue formation and

collagen deposition are seen. Healing processes in the hydrogel group (f), liposome group (g), nano-extract group (h), extract group (i) are similar to the negative control group.

Collagen deposition (green color) in burns in the different groups on the 10th day is shown in Figure 9.The amount of collagen deposition in the treatment group (c and d) and the positive control group (e) is higher than the negative control group (a and b), hydrogel group (f), liposome group (g), nano-extract group (h), extract (i).

Healing processes in burns in different groups on the 15th day are shown in Figure 10. As shown in this figure, In the negative control group (a and b) the re-epithelialization is incomplete. Large amount of immature granulation tissue with high cellularity and low collagen synthesis are seen. In the treatment group (c and d) complete re-epithelialization is seen. In the dermis mature granulation tissue with low angiogenesis and high organized collagen bundles are seen. In the positive control group (e) the healing processes are similar to the treatment group. In the nano-extract group (h), re-epithelialization is approximately completed but granulation tissue in the superficial part of dermis is immature. Healing processes in the hydrogel group (f), liposome group (g),

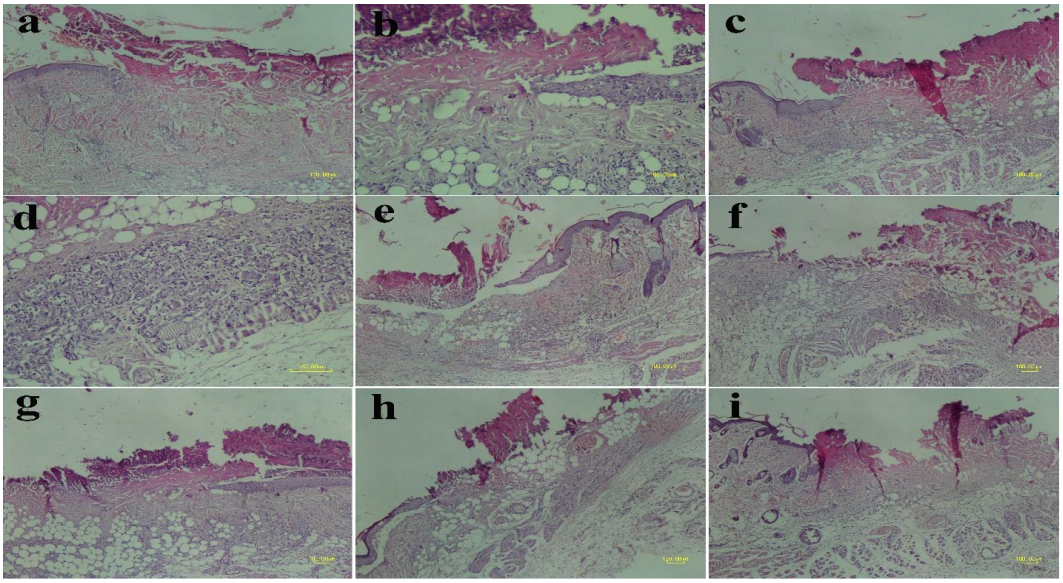

Figure 7. This photomicrograph shows healing processes in burns in different groups on the 5th days (H&E staining).

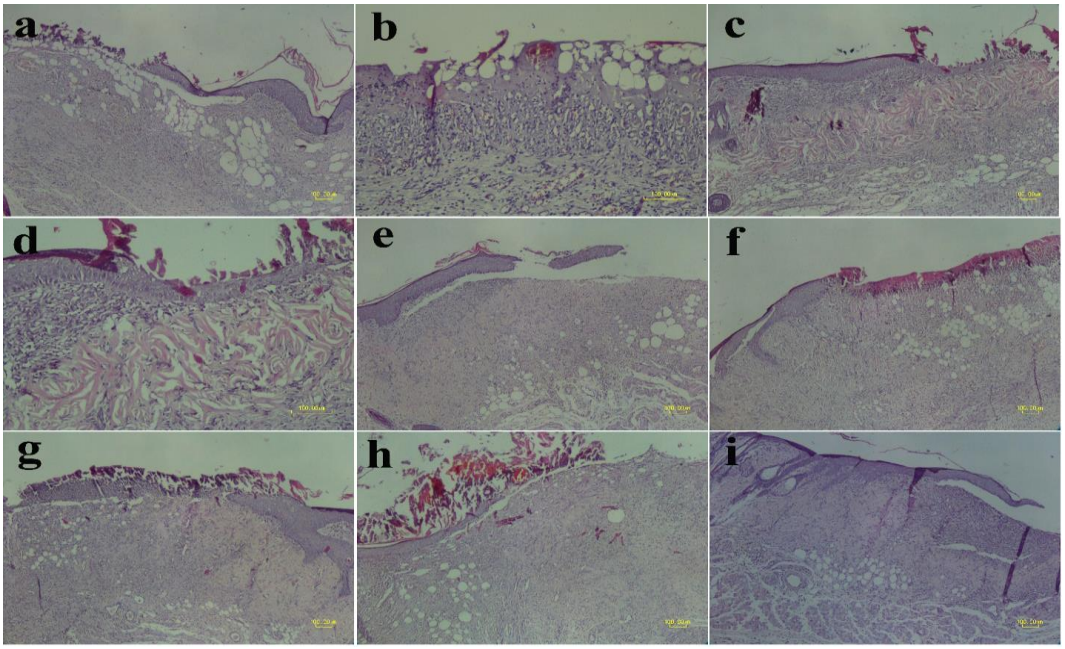

Figure 8. Photomicrograph healing processes in burns in different groups on the 10th days (H&E staining).

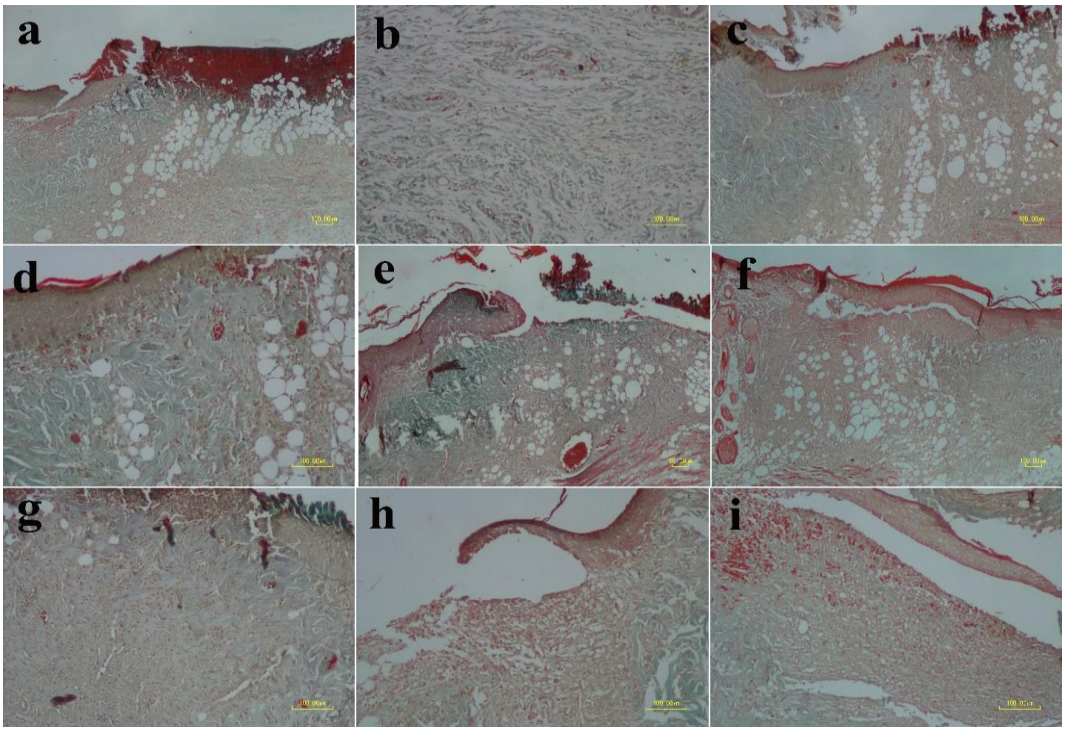

Figure 9. Photomicrograph reveals collagen deposition as green color in burns in different groups on the 10th day (Masson's trichrome staining).

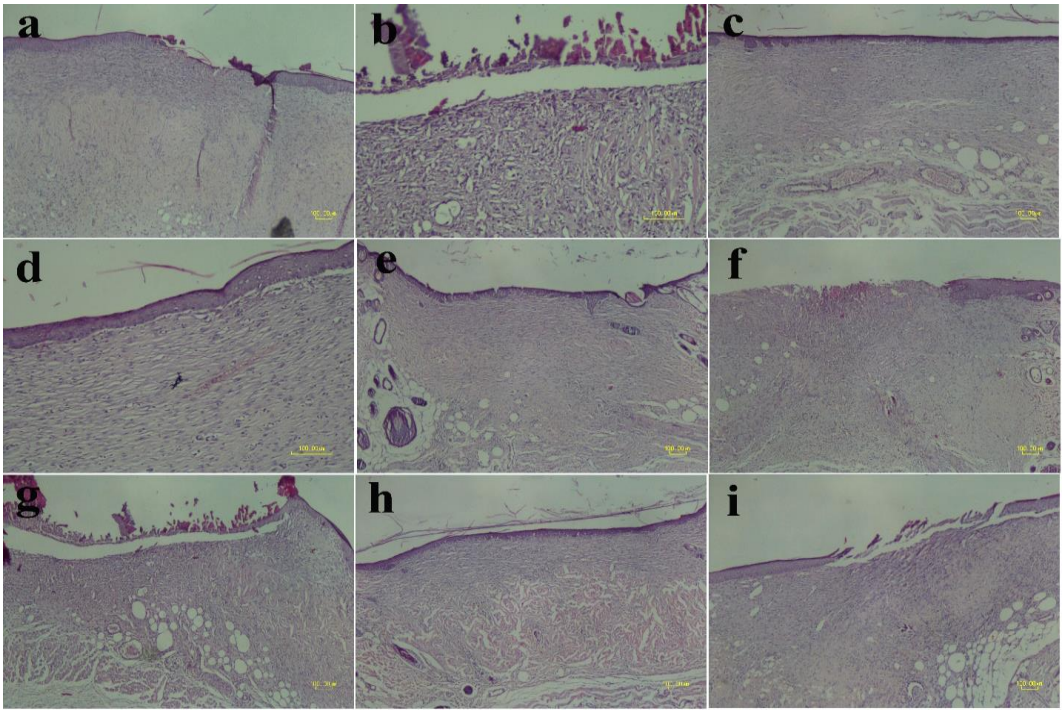

Figure 10. Healing processes in burns in different groups on the 15th day (H&E staining).

extract group (i) are similar to the negative control group.

Collagen deposition (green color) in burns in the different groups on the 15th day is shown in Figure 11. In the negative control group (a and b), hydrogel group (f), liposome group (g), extract group (i) low collagen deposition is seen. In treatment group (c and d) large amount of thick collagen bundles in the dermis along of the burned area are seen. In the positive control group (e) and the nano-extract group (h) the amount of collagen production is more in the margin of the wound and deep areas of the dermis.

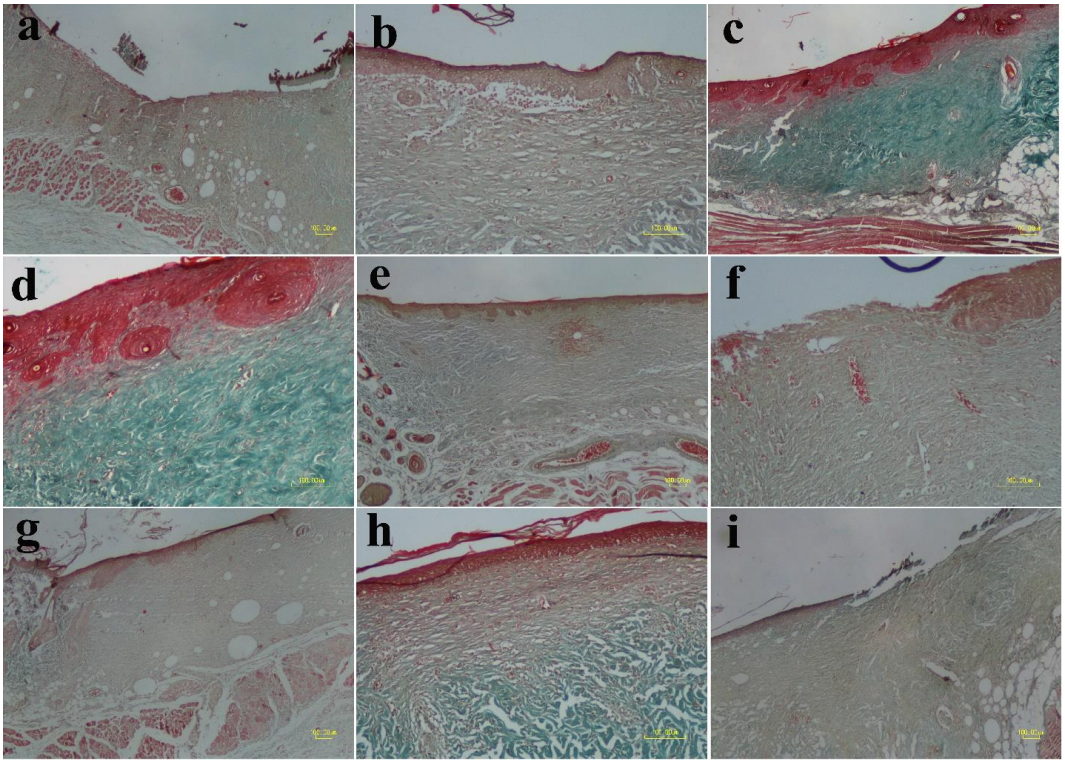

Figure 11. Photomicrograph reveals collagen deposition as green color in burns in different groups on the 15th day (Masson's trichrome staining).

Discussion

As the first barrier against the external environment and microorganisms, the skin plays an important role in protecting the host. Damage to the skin leads to the entry of microorganisms and other pathogenic agents that threaten the host's health.[2] Wound healing has different stages of inflammation, proliferation and reconstruction, each of which consists of many other stages, some of which interfere with each other and cannot be easily separated. The stages of wound repair and reconstruction include the hemostasis phase, inflammatory phase, proliferative phase, and remodeling phase.[38] The use of chemicals and antibiotics to treat and prevent wound infection may cause sensitivity, toxicity to the host, and the spread of antibiotic resistance in microorganisms, so the use of plant compounds as natural substances can be a method to prevent Harmful side effects.[8,39] *Scrophularia striata* is a plant that can be used in wound healing due to its anti-inflammatory and antioxidant properties.[40] Although the use of phytochemicals has limitations such as low solubility, low concentration, poor penetration into the skin and damaged area, nanoliposomes and hydrogel can reduce these limitations and deliver the drug to the damaged area.[41] Hydrogels are a natural and biological structure, and due to their network structure, they can absorb water and provide the necessary moisture for wound healing. Alginate and PEG can speed up wound healing by providing moisture and absorbing wound secretions. Based on previous findings, in this study, *Scrophularia striata* extract was prepared in the form of alginate-polyethylene glycol polymer hydrogel and its wound healing effect was investigated. In the present study, on the 5th day, there was a significant difference in the amount of acute inflammation and necrotic tissue between the treatment group and other groups, however no significant difference was observed between the treatment group and the zinc oxide group, which can indicate that the hydrogel containing *Scrophularia striata* extract has an acceptable effect and is equal to zinc oxide. The results of the present study was consistent with the study of Ali Ghashghaii *et al*.[40] and Mohammad Shahraki, *et al.*[35] On the 10th day, there was a significant difference in the formation of granulation tissue and vascularization between the treatment group and the control group. Although there was a significant difference between the treatment group and the extract group, this could be due to the presence of hydrogel and alginate-polyethylene glycol polymer. The anti-inflammatory effects and the organization of the granulation tissue performed by *Scrophularia striata* increase the speed of wound

healing and thus reduce the healing time.[40] Wound healing requires the supply of oxygen to the wound site. In this study, it is clear that wound healing has a direct relationship with the formation of new vessels, and the group treated with *Scrophularia striata* had a higher angiogenesis rate.[42] The results of this study are consistent with the previous findings and confirm the effect of *Scrophularia striata* in the formation of granulation tissue and vascularization.[17,35,40] On the 15th day, the amount of granulation tissue formation, the arrangement of collagens and angiogenesis among the treatment group showed a significant difference from other groups. It was also found that there is a significant difference between the treatment group and *Scrophularia striata* extract, which may be due to the presence of hydrogel based on alginate-polyethylene glycol polymer, which provides conditions for wound healing by providing moisture and water. It is possible that the increase in the number of collagen and its organization in the wound is due to the reduction of inflammatory reactions or the organization of the fibrin network, which is caused by the effect of *Scrophularia striata* extract.[43] The results of this study are consistent with previous studies.[35,40,44] In order to heal a wound, epithelial cells proliferate and migrate to the edges of the wound to cover it[42]. The results of the present study showed that on days 5, 10, and 15, the formation and rearrangement of epithelial tissue in the zinc oxide and treatment group were significantly different from other groups. Also, there was no significant difference between the zinc oxide group and the treatment group, and it indicates that *Scrophularia striata* hydrogel extract has the same effect as zinc oxide ointment. These results are consistent with previous studies that confirm the effect of *Scrophularia striata* extract on the formation and rearrangement of epithelial tissue.[40,44] As in previous studies,[35,45,46] in this study, it was found that *Scrophularia striata* hydrogel extract had the same effect as zinc oxide and had a significant difference from other groups in reducing the wound area. Reducing the wound area is influenced by angiogenesis, increasing oxygen supply to the wound, reducing inflammation, formation and regeneration of collagen, and on the other hand, *Scrophularia striata* extract can facilitate these processes and hydrogels provide the conditions for wound healing.[26,47] In the process of wound contraction, the skin around the wound moves and the wound is covered with the participation of communication between cells, extracellular matrix and cytokines.[45] The treatment group as well as the zinc oxide group had completely contracted the wound at the end of the treatment period and had significant differences from other groups. The results of this study confirmed the previous studies.[17,35] Also, the significant difference between the *Scrophularia striata* extract group and the treatment group may be due to the presence of hydrogel, which increased the conditions for the stability and durability of the *Scrophularia striata* extract, and by providing moisture, it caused complete healing of the wound.

In conclusion, *Scrophularia striata* extract has significant effects on wound healing, wound contraction, reducing inflammation, and increasing angiogenesis, on the other hand, hydrogels with their properties create suitable conditions for wound healing. The combination of *Scrophularia striata* extracts with hydrogels and biodegradable compounds such as polyethylene glycol alginate increase the effectiveness of this extract. This hydrogel extract can be used as a natural and biological compound in the treatment of wounds.

Conflict of Interest

The authors declare there is no conflict of interest.

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