Evaluation of the effects of curcumin nanoliposomes on viability and motility of fibroblast cells and burn wound healing in mice: an in vivo and in vitro study

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Background: Curcumin (diferuloylmethane) is one of the most active components of turmeric. This herbal compound has anti-inflammatory and positive wound-healing impacts. The principal objective of this study was to evaluate the impacts of curcumin nanoliposomes on cell viability and motility of mouse fibroblast NIH 3T3 cells and its wound healing effects on second-degree skin burns in BALB/c mice.

Methods: Mature male BALB/c mice (n = 48) were divided into 4 groups (n = 12 per group). Group one received curcumin nanoliposome ointment; the positive and negative control groups (groups 2&3) were treated with silver sulfadiazine and placebo, respectively, and group four (sham) received no treatment. The burn wound was created by a metal device with a diameter of 1 cm. Animals received treatment twice daily. On days 4, 7, 10, and 14, deep anesthesia and a biopsy of the wound were performed, and a microscopic study and MTT assay were carried out.

Results: Cellular studies on mouse fibroblast NIH-3T3 cells showed that low-dose curcumin nanoliposomes increased cell proliferation and motility at 8, 12, and 24 hours in comparison with the control group. In tissue samples of mice treated with curcumin nanoliposome (day 14), less inflammation was observed, while granulation tissue formation, fibroblast proliferation, epithelialization, and collagen fiber synthesis increased significantly compared with the control groups.

Conclusion: Our study indicates the positive effects of curcumin nanoliposomes on the motility process of mouse fibroblast NIH-3T3 cells (in vitro) and on the inflammatory and proliferative phases (in vivo) of burn wound healing in mice.

Keywords: nanoliposome, curcumin, wound healing, burn, fibroblast

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INTRODUCTION

The skin is a vital component of the body's inherent immunity, acting as a physical obstacle to prevent microorganisms, pathogens, and other chemicals from penetrating the body [1]. Morphologically, the skin is composed of the epidermis and dermis layers, made of packed epithelial cells and dense irregular connective tissue, respectively. The hypodermis also lies beneath the dermis and primarily comprises fatty tissues [2]. In case of any injury to the skin, this organ is disrupted, and a wound is induced.

Based on the time frame of healing, wounds are clinically categorized into two types: acute and chronic. Acute wounds are usually superficial and heal completely within 8 to 12 weeks, such as incisional wounds; chronic wounds last more than 12 weeks, including venous and arterial wounds [3,4].

Burning has been considered the fourth most common type of trauma worldwide, caused by different occurrences, such as traffic accidents, falls, and interpersonal violence [5]. In 2017, about 370,000 people in the United States had burn injuries, which shows a declining trend compared with 2008 (410,000 people) [6]. Burn wounds are caused by various factors, such as hot liquids, severe radiation, flames, electrical contact, and radiation [5]. Burns and injuries are major causes of death and disability worldwide. Besides, these types of injuries are the main cause of temporary and significant scars on the body. Such injuries, ignoring the form of treatment and costs, could lead to abnormalities such as isolation or depression. Local and systemic burn injuries reduce the skin's resistance as a barrier to the environment and impede its ability as an immune organ [7].

The natural process of wound healing is set by a number of cellular and chemical responses in the body, specifically at the wound site, beginning immediately after the creation of the wound. This process is formed with a very complex and developed mechanism resulting from various overlapping steps such as hemostasis, inflammation, proliferation, and remodeling [8,9]. In the hemostasis phase, the arteries are constricted, and platelets activate the coagulation process. In the inflammatory phase, neutrophils and then lymphocytes migrate to the wound site, and their main activity is to prevent infection. Macrophages are the most important cells in completing the inflammatory phase. The proliferation phase, the third phase, involves the formation of new blood vessels, fibroblast proliferation, and collagen synthesis; together, they create the granulation tissue that helps with epithelium formation. In the remodeling phase, fibroblasts can deposit new collagen fibers, and in each fiber, the fibril packing density increases, resulting in a collagen fiber with a larger diameter and stronger mechanical properties [9,10].

Despite extensive development in wound healing, the use of traditional methods of wound healing and the use of herbal medicines and their derivatives has increased in recent decades, and extensive research on the effectiveness, properties, and dosage of these compounds has led to the growing use of herbal compounds in the wound healing process [11]. Recent studies have shown that in burn healing, other than using plants, we can use animal fat oil [12]. Turmeric has been used as a type of spice for centuries, and curcumin, with strong antioxidant activity, is one of the active components of this plant. Its verified biological properties include anti-cancer, anti-angiogenesis, anti-metastatic, anti-inflammatory, anti-Alzheimer, and antioxidant properties [13,14].

The use of curcumin is somewhat limited because of its rapid metabolism, low bioavailability, poor solubility, photosensitivity, and degradation under alkaline conditions [15]. For this reason, nanotechnology can increase its efficiency. Effective wound care treatments should include multidimensional approaches to deal with complex wound problems, such as pain, inflammation, and infection. Novel nanoparticle-based therapies may improve wound and burn healing by increasing contact level, adsorption, bioavailability, and persistence of active agents. Generally, nanotechnology is used to enhance drugs' power and efficiency, reduce side effects, and direct drugs to the given tissues [16]. Nanoliposomes are colloid particles composed of lipid molecules; after reacting with water, nanoliposomes accumulate in the bilayer membrane and are converted into a spherical shape with energy and sheer force. Liposomes are hydrophilic and can enclose the active hydrophilic material inside and on the surface, with the hydrophobic materials inside the membrane [17]. Hence, we aimed to evaluate the impacts of curcumin nanoliposomes on the viability and cell motility of mouse fibroblasts in a cell culture medium and on repairing burn wounds of BALB/c mice.

PARTICIPANTS AND METHODS

Preparation of experimental animals

Male mature BALB/c mice were used in this experimental study. Animals were provided by the Experimental Medicine Research Center of Birjand University of Medical Sciences, Birjand, Iran. Mice (2.5 months of age) were kept in separate

cages to be adapted to the new environment. Environmental conditions were controlled in terms of temperature (25 °C), humidity (50%), and light (12:12 h light-dark cycles). Mice had access to standard laboratory chow. The drinking water was supplied from the city's tap water. The cages were cleaned every two days, and the extra food was replaced with fresh food.

Indicators measuring method

Initially, 48 healthy male BALB/c mice were kept in cages in an appropriate environment. Anesthetization in mice was performed by intraperitoneal (IP) injection of ketamine 70 and xylazine 10 mg/kg. The hair of the dorsal thoracic skin was completely shaved. Then, by using a metal device with a rod and a flat circular head one centimeter in diameter, a superficial burn wound (grade 2) in the form of a 1 cm diameter circle, including the thickness of the dermis and hypodermis, was created. Animals were randomly divided into four groups. In the first group, mice received curcumin nanoliposome ointment (treated group); the second group (as a positive control) received 1% silver sulfadiazine topical cream, and the third group (as a negative control) received a curcumin-free nanoliposome ointment as the placebo. The fourth group (sham) was not treated. All groups except the sham group were treated twice daily at 8 am and 8 pm. On days 4, 7, 10, and 14 after treatment, 3 mice from each group were anesthetized, and skin samples were taken for histological evaluations.

Histological analysis

Tissue samples were fixed in 10% formaldehyde solute, dehydrated with excessive alcohol, and cleared in xylene during tissue passage steps. Then, they were embedded in paraffin, and 5 µm sections were prepared using a microtome. Finally, sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome techniques. Finally, two blinded pathologists assessed the slides under a light microscope. For each sample, epidermal parameters (regeneration) and dermal parameters (inflammatory cells, granulation tissue, and collagen fiber density) were examined. Image processing program software (Image J, NIH) was used for histological analysis of the images.

Nanoliposomal curcumin ointment

Topical nanoliposomal curcumin (1% w/w) with the trade name Sinanomin™ and placebo cream (base without curcumin) was provided by ExirNanoSina Company, Tehran, Iran. These nanoliposomes had an average particle diameter of 100 nm, and their encapsulation efficiencies for curcumin were more than 99%.

MTT assay for cell viability

To determine the in vitro cytotoxicity of the curcumin nanoliposomes on fibroblast NIH 3T3 cells, the MTT test was performed. Cells (1×10^4 cells/well) were treated with a serum-free culture medium of curcumin nanoliposomes (7.81-125 ug/ml) after 24 h incubation. Each well received about 20 µl of MTT reagent (5 mg/ml) and was incubated at 37 ℃ for another 4 h. Then, the media were removed, and formazan crystals were dissolved in 100 µl of dimethyl sulfoxide (DMSO). By using the Epoch Microplate Spectrophotometer (Biotek Instrument, USA), solubilized formazan absorbance was measured at 570 and 630 nm. Cells incubated in the control medium were considered 100% viable.

Scratch wound healing assay

A scratch wound-healing assay was used to assess the proliferation/migration capabilities of fibroblast NIH 3T3 cells. The cells were seeded in a 24-well plate in a humidified atmosphere containing 5% CO_2 at a concentration of 2×10^5 cells/mL. Next, the cells were cultivated in a medium containing 10% of FBS to form a confluent monolayer. Then, to create a wound, monolayer cells were scratched in a straight line pattern using a sterile 100 µl pipette tip. PBS solution and a serum-free medium were used to remove the cell debris and smooth the scratch edge, respectively. Images were immediately collected, labeled (0 h), and incubated with 250 µg/ml and without (as a control) curcumin nanoliposomes for one day at 1% O_2 and 5% CO_2. Any reduction in the scraped region was considered as wound healing and migration of the cells. To quantify the scratch closure, two-time periods, time 0 and time 24 h, were determined, and the difference between wound width was calculated. To allow identification of

the same scratched area to take consistent pictures, each well was marked below the plate surface by drawing a vertical line. Finally, image J software (National Institutes of Health, Bethesda, MD, USA) was used to measure the scratch area. The migration rate was calculated according to the following equation: scratch closure rate = (scratch width at 0 h - the remaining scratch width at 24h) / scratch width at 0 h × 100.

Statistical studies

Quantitative parameters of collagen fibers, epithelialization, and granular tissue size were measured by Image J software. Quantitative data were reported as the mean ± standard error of the mean (SEM). Analysis of the data was performed using SPSS statistical software (version 22). One-way analysis of variance (ANOVA) and Tukey's test were applied to compare the results of the means between groups. $P < 0.05$ was considered statistically significant.

Ethical considerations

The ethical standards of working with laboratory animals were upheld throughout the work. The study protocol was approved by the Ethics Committee of Birjand University of Medical Sciences (IR.BUMS.REC.1399.267)

RESULTS

In vitro studies of curcumin nanoparticles

1A-Cell viability assay. The cytotoxicity of curcumin nanoliposomes on mouse fibroblast NIH-3T3 cells was evaluated at different concentrations ranging from 125 to 7.81 ug/ml at 24 and 48 hours using the MTT technique. The results are presented in Figure 1. It was found that in a time-dependent manner, the cell viability decreased with increasing concentrations. However, at concentrations less than 15.62 µg/ml of curcumin nanoliposomes, no toxic effects on cell viability were observed. Therefore, the concentration of 7.81 µg/ml was selected for further cell studies.

1B-Scratch assay. As shown in Figures 2 and 3, the group treated with media containing 7.81 µg/ml of curcumin showed faster gap closure of scratched cells than the control group with media alone at 8, 12, and 24 hours. While at 36 ($P = 0.208$) and 48 ($P = 0.185$) hours, no significant difference in gap closure was seen between the treated and control groups.

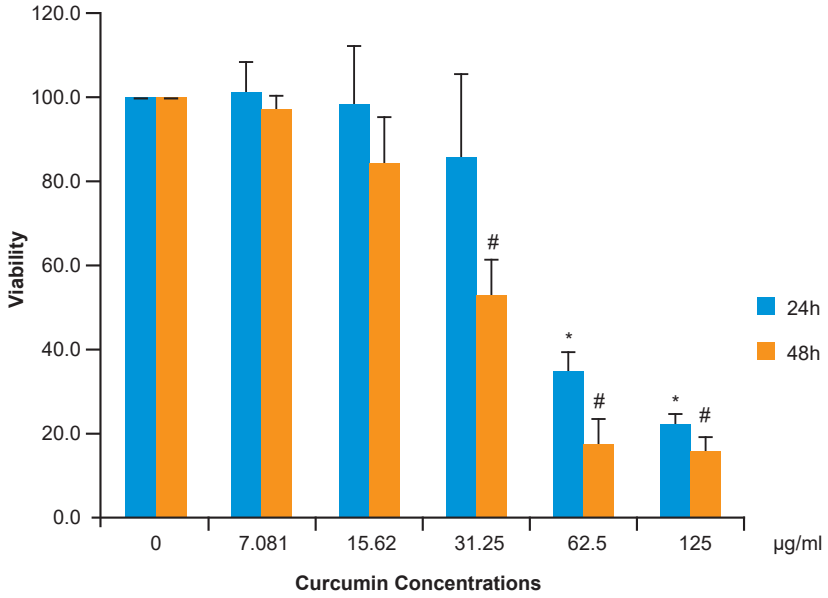

Figure 1. The effect of different concentrations of curcumin nanoparticles on the viability of mouse fibroblast NIH-3T3 cells. *A significant difference (P < 0.001) compared with the control group at 24 hours. #A significant difference (P < 0.001) compared with the control group at 48 hours. Kruskal-Wallis statistical test (n = 4)

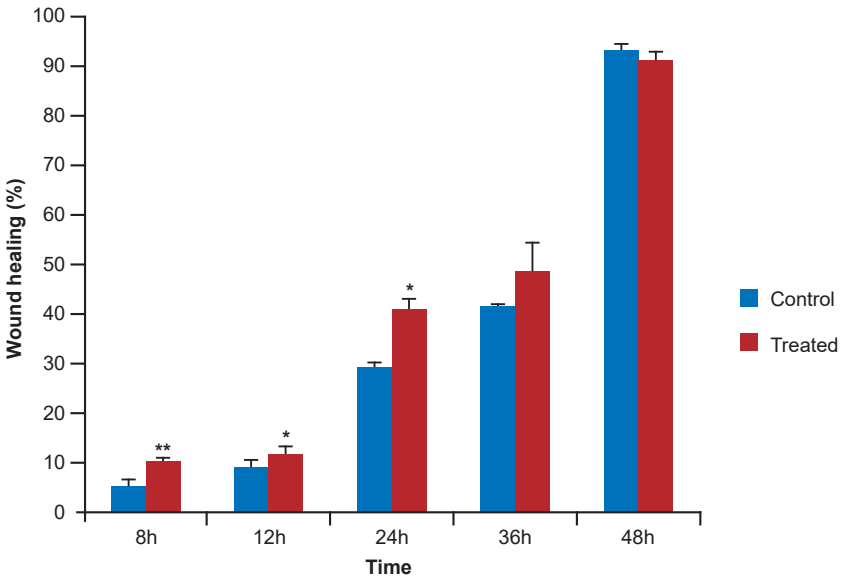

Figure 2. The effect of curcumin nanoparticles (7.81 µg/ml) at different times on mouse fibroblast NIH-3T3 cells' motility.

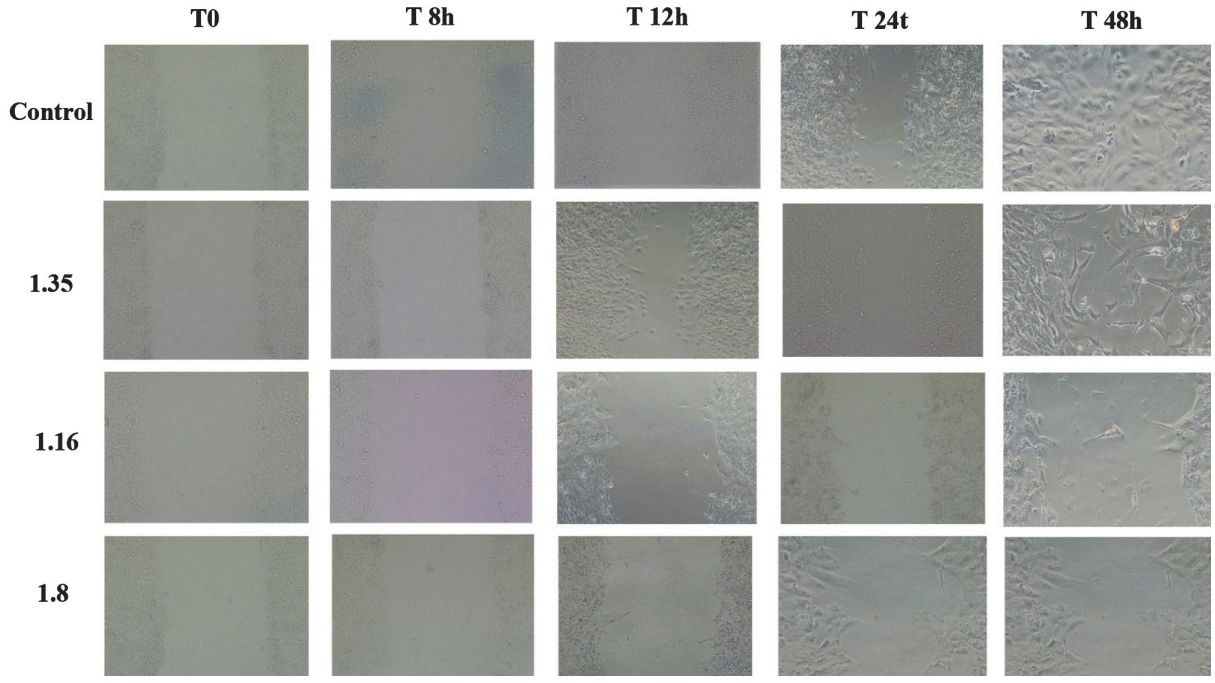

Figure 3. The images of the scratch test at different times.

In vivo studies of curcumin nanoparticles

This study's results indicate the useful impacts of curcumin nanoparticles in different steps of wound healing, namely inflammatory and proliferation phases in vivo (Table 1 and Figure 4).

2A. Evaluation of inflammatory cells. In the evaluation of the mean of inflammatory cells on days 4, 7, and 10, the results are as follows (Table 1): on the fourth day, the total number of inflammatory cells in the group treated with curcumin nanoliposomes and the silver sulfadiazine group showed a significant decrease ($P < 0.001$) compared with both placebo and

Table 1. Microscopic data of wounds in the treatment group and other control groups on different days of the study

Parameter	Day	CUR	Silver	Placebo	CONTROL	ANOVA
Total Inflammatory Cells	Day 4	41.50 ± 1.77 *,**,#	45.90 ± 1.59 *,#	49.10 ± 1.52	53.1 ± 1.59	P<0.001 F=91.31
	Day 7	45.20 ± 1.82 *,**,#	53.60 ± 4.11 *,#	57.90 ± 4.20	61.30 ± 3.30	P<0.001 F=38.78
	Day 10	38.3 ± 1.88 *,**,#	42.30 ± 1.05 *,#	49.90 ± 1.79	52.40 ± 1.71	P<0.001 F=158.582
Fibroblast Cells	Day 7	32.70 ± 1.63 *,**,#	29.00 ± 1.24 *,#	26.40 ± 0.96	25.00 ± 1.05	P<0.001 F=72.95
	Day 10	36.70 ± 1.05 *,#	36.40 ± .96 *,#	26.60 ± 1.07	25.20 ± 1.03	P<0.001 F=356.719
	Day 14	39.70 ± 1.33 *,**,#	36.90 ± 1.28 *,#	33.90 ± 1.52	32.90 ± 1.79	P<0.001 F=42.223
The Rate of Granulation	Day 7	227788.6 ± 35198.97 *,#	194736.10 ± 30455.79	183998.3 ± 23897	186067.20 ± 30027.69	P<0.009 F=4.53
	Day 10	272091.8 ± 49902.81 *,**,#	192406.7 ± 39482.15	171415.6 ± 46177.63	167212.30 ± 23798.49	P<0.001 F=14.117
	Day 14	326739.90 ± 57087.11 *,**,#	239125.30 ± 25221.49	223324.10 ± 38340.31	213611.60 ± 32624.89	P<0.001 F=16.675
Collagen Fiber Density	Day 10	0.3278 ± 0.0121 *,**,#	0.3040 ± 0.01430 *,#	0.2820 ± 0.01033	0.2620 ± 0.01033	P<0.001 F=56.865
	Day 14	0.355 ± 0.013 *,#	0.346 ± 0.012 *,#	0.324 ± 0.010	0.316 ± 0.009	P<0.001 F=24.211
Epithelialization	Day 10	304.31 ± 49.96 *,**,#	192.22 ± 31.68 *	177.10 ± 42.88	134.96 ± 32.95	P<0.001 F=32.534
	Day 14	380.09 ± 18.958 *,**,#	335.92 ± 20.549 *,#	295.03 ± 3.960	236.47 ± 31.316	P<0.001 F=83.988

*P < 0.05: compared with the control group. **P < 0.05: compared with the silver group. #P < 0.05: compared with the placebo group.

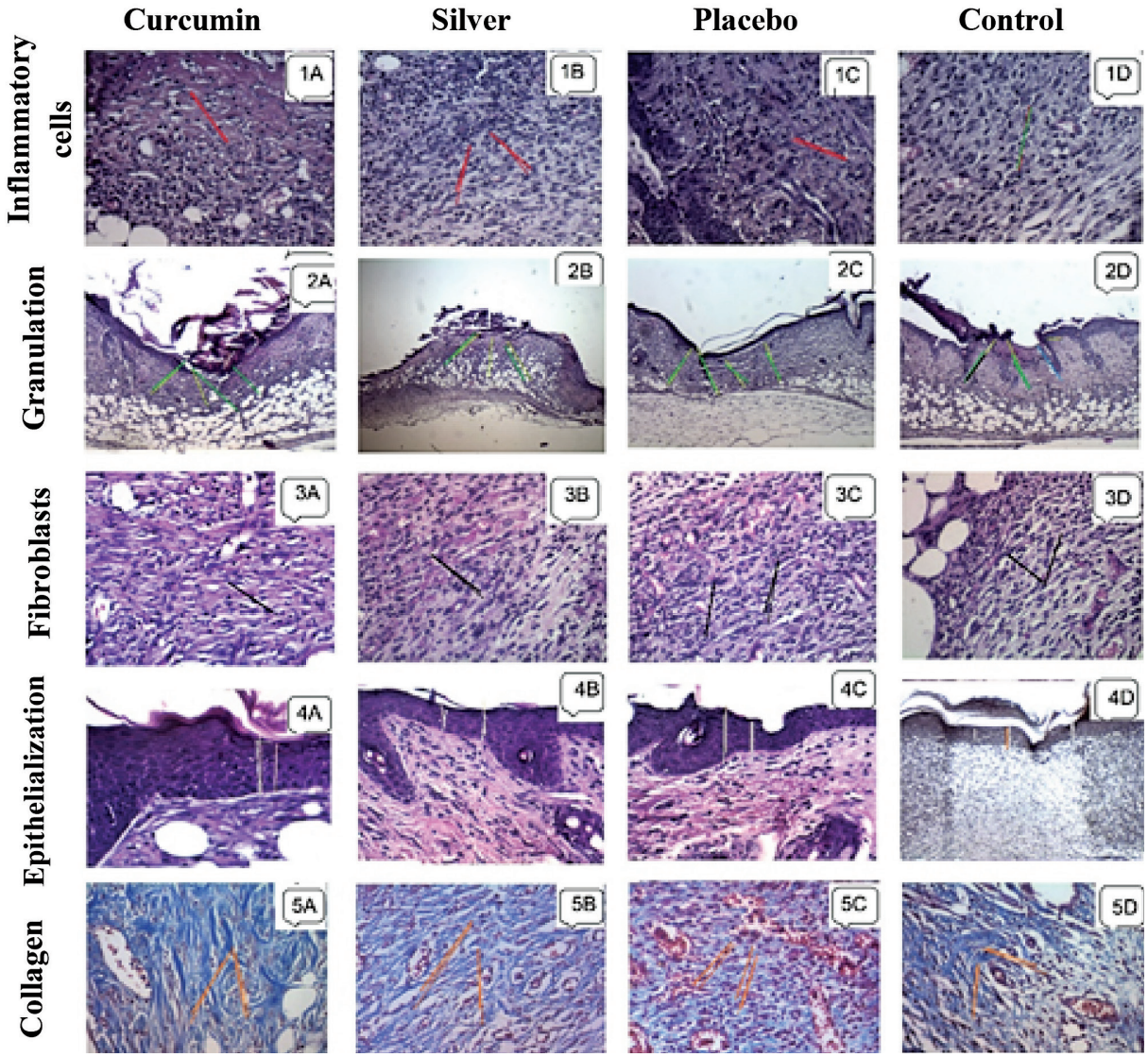

Figure 4. Microscopically evaluated parameters on different days of this study: (1a-d) Inflammatory cells on day 7 (red arrows) (H&E, 100×). (2a-d) Granular tissue area on day 10 (green arrows) (H&E, 40×). (3a-d) Number of fibroblasts on day 10 (black arrows) (H&E, 100×). (4a-d) Epithelialization on day 14 (white arrows) (H&E, 100×). (5a-d) Density of collagen fibers on day 10 (orange arrows) (Masson's trichrome stain, 100×).

sham control groups. The average total number of inflammatory cells in the group treated with curcumin nanoliposomes showed a remarkable difference ($P < 0.001$) in comparison with the silver sulfadiazine group.

The results on the seventh day showed that the average total number of inflammatory cells in the group treated with curcumin nanoliposomes and the silver sulfadiazine group was significantly different in comparison with the placebo and sham control groups ($P < 0.001$). In addition, the average total number of inflammatory cells in the group treated with curcumin nanoliposomes was significantly reduced in comparison with the silver sulfadiazine group ($P < 0.001$).

On the tenth day after the intervention, findings similar to days 4 and 7 were repeated, and the lowest mean number of inflammatory cells was observed in the group treated with curcumin nanoliposomes. This group's mean was significantly lower than the silver sulfadiazine group ($P < 0.001$).

2B. The number of fibroblasts and granular

tissue formation. On days 7, 10, and 14 of this study, microscopic evaluations were performed on the mean number of fibroblasts and the area of granulation tissue formation.

Obtained results showed that on the seventh day after the intervention, there was a significant increase ($P < 0.001$) in the mean number of fibroblasts in the group treated with curcumin nanoliposomes and silver sulfadiazine in comparison with both placebo and sham control groups. The average total number of fibroblasts in the group treated with curcumin nanoliposomes was significantly ($P < 0.001$) higher than in the silver sulfadiazine group.

On the tenth day after the intervention, the mean number of fibroblasts in the groups treated with curcumin nanoliposomes and silver sulfadiazine showed a remarkable increase ($P < 0.001$) in comparison with the placebo and sham control groups, but there was no significant difference in the group treated with curcumin nanoliposomes in comparison with the silver sulfadiazine group in terms of the average total number of fibroblast cells.

The results showed that on the fourteenth day after the intervention, there was a remarkable increase ($P < 0.001$) in the mean number of fibroblasts in the groups treated with curcumin nanoliposomes and silver sulfadiazine in comparison with the placebo and sham control groups. Also, the mean number of fibroblast cells in the group treated with curcumin nanoliposomes had a significant increase ($P < 0.001$) compared with the silver sulfadiazine group.

The mean area of granulation tissue in the group treated with curcumin nanoliposomes on the seventh day increased significantly ($P < 0.009$) in comparison with the placebo and sham control groups. The mean area of granular tissue in the group treated with curcumin nanoliposomes did not show any significant difference from the silver sulfadiazine group.

On the tenth day, there was a remarkable increase in the mean area of granular tissue in the group treated with curcumin nanoliposomes in comparison with the placebo and sham control groups. Furthermore, the mean area of granulation in the group treated with curcumin nanoliposomes was remarkably different ($P < 0.001$) in comparison with the silver sulfadiazine group. The mean granulation in the group treated with curcumin nanoliposomes on the fourteenth day showed a significant increase ($P < 0.001$) in comparison with the silver sulfadiazine, placebo, and sham control groups.

2C. Collagen fibers' density and epithelialization. Findings of this study regarding the collagen fibers' density and epithelialization on days 10 and 14 after the intervention are presented as follows: on the tenth day after the intervention, the mean density of collagen fibers in the groups treated with curcumin nanoliposomes and silver sulfadiazine showed a remarkable increase ($P < 0.001$) in comparison with both placebo and sham control groups. In addition, the mean density of collagen fibers in the group treated with curcumin nanoliposomes showed a significant difference ($P < 0.001$) in comparison with the silver sulfadiazine group.

The results showed that on the fourteenth day after the intervention, the mean density of collagen fibers in the groups treated with curcumin nanoliposomes and silver sulfadiazine showed a significant increase ($p < 0.001$) in comparison with both placebo and sham control groups. No significant difference was observed in the group treated with curcumin nanoliposomes in comparison with the silver sulfadiazine group.

The mean rate of epithelialization on the tenth and fourteenth days was similar and showed a remarkable increase in the groups treated with curcumin nanoliposome or silver sulfadiazine in comparison with both placebo and sham control groups. There was a significant difference ($P < 0.001$) in the mean epithelialization of the group treated with curcumin nanoliposomes compared with the silver sulfadiazine group.

DISCUSSION

In this experimental study, the impacts of curcumin nanoliposomes on the healing of second-degree burn wounds in BALB/c mice and mouse fibroblast NIH-3T3 cells were investigated. Our findings show the useful effects of this substance in different steps of wound healing, namely the inflammatory and proliferation phases in vivo. Also, the in vitro studies on mouse fibroblast NIH-3T3 cells showed proliferation and increased cellular motility of fibroblasts under the influence of curcumin nanoliposomes.

The effects of curcumin on the inflammatory phase of wound healing

In the inflammatory phase, which begins immediately after blood vessel damage and leakage of material from the blood vessels, small arteries dilate due to the activation of the complement system, and plasma proteins and white blood cells enter the wound site. In addition to macrophages, neutrophils and lymphocytes are among the most important cells that migrate to the wound site, and their main activity is to prevent infection [18].

In our study, inflammation and infiltration of neutrophils and lymphocytes on the studied days [4,5,9] were less in the curcumin nanoliposome-treated group than in the control groups, and these nanoparticles showed even better anti-inflammatory effects than silver sulfadiazine. A number of studies have been performed to confirm the positive effects of curcumin nanoliposomes on inflammation control, indicating that these nanoliposomes can prevent excessive inflammation. One study evaluated the effects of curcumin, chrysin, and a combination of chrysin–curcumin-loaded PCL-PEG nanofibres on the process of excisional wound healing in male rats. The results of the mentioned study showed that chrysin–curcumin-loaded PCL-PEG nanofibres could affect the levels of IL-6, MMP-2, TIMP-1, and, by affecting the expression of TIMP-2 and iNOS genes, could produce good anti-inflammatory activity in the wound healing process. One of the important results of that study was the dose-dependent effects of these compounds in the control of wound inflammation [19]. Another study by Fang *et al.* investigated the effects of curcumin-loaded chitosan nanoparticles on excisional wounds on the skin of diabetic mice by STZ. Although diabetes disrupts the wound healing process and increases inflammation, these nanoparticles were able to decrease the inflammation caused by the wounds. Interestingly, these nanoparticles were able to effectively attenuate the macrophage-mediated inflammation resulting from its entrance into the wound site and enhance angiogenesis [20]. The results of the present study are also consistent with several previous findings, and it can be assumed that the significant anti-inflammatory effects of curcumin nanoliposomes can accelerate wound healing by shortening the inflammatory phase.

The important point here is the probable mechanisms of inflammation reduction by curcumin. Recent mechanistic studies have largely revealed the possible mechanisms of the effects of this material on suppressing the inflammation process, which we will briefly consider. Some recent research has shown that curcumin is a highly functional molecule during the inflammatory process, reacting with various molecules involved in the inflammatory phenomenon. Curcumin affects the inflammatory process by inactivating cyclooxygenase (COX-2), lipoxygenase, and inducible nitric oxide synthase (iNOS) enzymes. Studies also show that curcumin can apply its anti-inflammatory effects by inhibiting the inflammatory mediators of TNF-α cytokines and affecting the incidence of interleukin-1 and inactivation of NF-κB. The monocyte chemical reactive protein (MCP) is also an important inflammatory mediator affected by curcumin. This mediator can control the inflammatory process as it can affect the infiltration and migration of monocytes and macrophages [21]. One study showed that curcumin, by reducing the migration and infiltration of inflammatory cells, can reduce the MCP1 level, thereby suppressing inflammation [22].

Effects of curcumin on the proliferative phase of wound healing

The proliferative phase involves the formation of new tissue via the activation and migration of fibroblasts; production of glycosaminoglycans, proteoglycans, and fine collagen fibers by fibroblasts; differentiation of fibroblasts into myofibroblasts; angiogenesis; and epithelialization. The onset of fibroblast migration into the fibrin mass and the formation of new capillaries in the young fibroblast tissue cause the formation of granular tissue, which provides a good environment for the migration and proliferation of epithelial cells [23,24]. Therefore, fibroblasts have a crucial role in the wound healing process and can be regarded as the most important cells in the proliferative phase of wound healing. The speed of wound closure, the synthesis of collagen fibers, and the formation of granulation tissue are the most essential activities of fibroblasts. Studies on the effects of curcumin on fibroblast activity can be categorized into in vivo and in vitro studies, which sometimes do not provide the same results. Here, we first consider the in vivo studies of curcumin on the activity and migration

of fibroblasts and the formation of collagen and granular tissue. In a study by Dehghani *et al.*, the effect of curcumin on the formation of new epithelial tissue and angiogenesis in diabetic rat wounds was examined, and it was found that curcumin had a positive effect on the processes related to wound healing [25]. Alibolandi *et al.* demonstrated that curcumin nanomicelles incorporated with dextran hydrogel could significantly increase re-epithelialization, fibroblast deposition, and collagen formation in excisional wounds in BALB/c mice [26].

The mentioned in vivo studies confirmed curcumin's positive effects on the migration, proliferation, and differentiation of fibroblasts and the formation of collagen fibers and granulation tissue. However, in relation to the in vitro effects of curcumin on the activity of fibroblasts, the following points might be mentioned:

Some studies indicate that curcumin increases reactive oxygen species (ROS) in vitro, thereby increasing apoptosis in fibroblast cells. One study explored the potential antifibrotic impact of curcumin and NAC (N-acetylcysteine as a precursor to the antioxidant glutathione) individually and in combination. In that study, two sources of cells including primary epithelial cells and fibroblasts isolated from patients with idiopathic pulmonary fibrosis (IPF) were treated with a combination treatment of NAC and curcumin. Although treatment with curcumin had an antifibrotic potential, this effect was associated with increased oxidative stress. On the other hand, treatment with NAC showed that although the level of oxidative stress was reduced, the viability of epithelial cells was also decreased. But, simultaneous treatment with the two mentioned substances not only could decrease oxidative stress but also could maintain high viability in cells [27]. Another study evaluated the possible protective role of curcumin against sodium arsenite toxicity (0.01–10 μM) in 3T3 fibroblast cells for 24 hours [28]. Sodium arsenite, by enhancing the amount of hydrogen peroxide, lipid peroxidation, as well as hydroxyl radicals in 3T3 cells, could induce oxidative damage, while treatment with curcumin via its antioxidant properties could protect fibroblast cells against cytotoxicity resulting from sodium arsenite [28]. In another study by Sukumaran *et al.*, a wound healing or scratch assay was performed to compare the effects of curcumin and chlorhexidine on fibroblast migration. In line with our findings, curcumin nanoliposomes at a concentration of 0.015% positively affected fibroblast migration compared to higher concentrations of curcumin nanoliposomes (0.03 and 0.06%). Their study also stated that a concentration of 0.003% of curcumin increased fibroblast migration [29].

Based on all these studies, it can be said that the dose of curcumin can cause different results in fibroblast cell activity and even cause apoptosis of these cells, so this issue should be considered. On the other hand, under in vitro conditions, many effective factors in vivo of the cell environment (cytokine and the effects of extracellular matrix) are eliminated and can produce different results regarding the effects of curcumin on the activity and proliferation of fibroblast cells.

CONCLUSION

Our study shows that curcumin nanoliposomes can increase the proliferation and migration of mouse fibroblast NIH-3T3 cells in vitro. These nanoliposomes also positively affected the healing process of second-degree burns in BALB/c mice. Good anti-inflammatory effects in the inflammatory phase of healing and positive effects on fibroblast cell proliferation, collagen fiber regeneration and epithelialization, and granulation tissue formation during the proliferative phase render this compound an appropriate candidate for treating this type of burn. Therefore, further studies in the clinical trial phase are recommended.

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Conflict of Interest: None declared.

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