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ORIGINAL ARTICLE

Evaluation of propylene glycol nanoliposomes containing curcumin on burn wound model in rat: biocompatibility, wound healing, and anti-bacterial effects

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Abstract Curcumin is an effective wound healing agent in burn therapy, but due to its low bioavailability, it is required to be formulated for topical therapy. Liposomal nanocarriers are developed as stable and efficient dermal delivery systems. In this study, we prepared curcumin-propylene glycol liposomes (Cur-PgL) to treat animals subjected to second degree burns. The characterization tests confirmed the production of monodisperse nanoliposomes of average size of about 145 nm with high entrapment efficiency percentage and a sustained release behavior. TEM analysis of nanocarriers showed no aggregation in long time storage up to 60 days. The biocompatibility of the Cur-PgL formulation was evaluated by ISO standards. We found that Cur-PgL 0.3% was the effective dose in injured rats without any side effects on intact skin. The cytotoxicity of the Cur-PgL 0.3% nanovesicles was also assessed on human dermal fibroblast (HDF) cells. The results showed no detectable cytotoxicity, but considerable cytotoxicity was observed in higher concentration of 1.5 and 3 mg/ml of free and PgL forms of curcumin. Eight days of application of Cur-PgL on burned rats resulted in a significant ($P<0.001$) recovery of wound repair parameters, and after 18 days, wound contraction occurred significantly ($P < 0.001$) compared to the other groups. The antibacterial activity of the Cur-PgL formulation was found to be similar to the silver sulfadiazine (SSD) cream 1% regarding the inhibition of the bacterial growth. In conclusion, the low dose of curcumin nanoliposomal formulation efficiently improved injuries and infections of burn wounds and it can be considered in burn therapy.

Keywords Burn infection · Wound healing · Curcumin · Nanoliposome · Propylene glycol · Biocompatibility

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Introduction

Burns are one of the most painful forms of injuries that can affect various organs and systems depending on their type and severity. Despite many advances in the treatment and the management of burn injury, there is still a huge demand for novel therapeutic agents in burn therapy [1]. The main approach in burn therapy is to promote wound healing while managing burn wound infections [2]. Hence, multifunctional compounds such as curcumin may be a good candidate to improve the burn therapy outcome [3–5].

Curcumin obtained from the dried rhizomes of *Curcuma longa* is known as a natural polyphenol compound and has been used in indigenous medicine for centuries [3, 6]. Due to its specific structure and characterization, extensive studies have been done to reveal its various biological activities and therapeutic effects in recent years. Several studies have provided scientific

evidence regarding the anticancer, antioxidant, anti-inflammatory, and wound healing of curcumin [4, 7]. Curcumin also has a broad spectrum of antimicrobial activities against different bacteria, viruses, fungi, and parasites [3]. In spite of all these advantages, curcumin has poor bioavailability due to low water solubility, rapid metabolism, and rapid elimination and low stability. Several vesicular systems have been developed to overcome this limitation such as ethosomal and liposomal carriers [8, 9]. Liposome nanocarriers were developed for sustained release and enhanced transdermal delivery of different types of molecules to overcome the possible drug overdose and the toxicity as well as increasing the drug efficiency [8, 9].

Recent reports showed that nanosized liposomes containing propylene glycol (Pg) may be introduced as a novel dermal delivery carrier due to their elevated flexibility, good stability, and biocompatibility [8–12]. Pg is widely used as a penetration enhancer and emulsifier in topical formulations [10, 12]. Modification of liposomes with Pg provides deformable nanovesicles with enhanced permeation and retention properties in skin layers [8]. A recent study on curcumin liposomes containing Pg formulations showed that it was possible to deliver several-fold higher drug concentrations into the skin layers by vesicular systems with longer retention time compared to the free form. The results showed that curcumin-loaded Pg liposomes had the highest and longest anti-inflammatory effect on the development of rat paw edema compared to ethosomes and traditional liposomes [8].

The purpose of this study is to evaluate the curcumin-loaded Pg nanoliposomes in skin burn therapy. At first, the formulation was optimized by dose response assessment via wound assessment protocols. Then, the biocompatibility of the optimized concentration of curcumin nanoliposomes was assessed by the ISO10993-10 standard. The neutral red uptake assay as a cell cytotoxicity test was also applied for the safety evaluation of the formulation on human dermal fibroblast cells. We also determined the physical characterizations of nanoliposomal curcumin including the shape, size, loading capacity, and encapsulation efficiency as well as in vitro release profile. The biocompatibility and efficiency of the Cur-PgL 0.3% formulation including safety, antibacterial activity, and wound healing on a burn model could be considered as beneficial factors in translational medicine.

Materials and methods

Materials

Soy lecithin was obtained from Daejung (Sought Korea). Formaldehyde, ethanol, and methanol were purchased from Merck (Germany). Curcumin, cholesterol, and propylene glycol were purchased from Sigma (USA). Silver sulfadiazine (SSD) cream (1% *w*/*w*) was obtained from Sobhan Darou Company (Iran). Neutral red solution was obtained from Sigma (St. Louis, MO, USA).

Curcumin nanoliposome preparation and characterization

A procedure with nontoxic chemicals and fast production was used for nanoliposome preparation [12]. Briefly, a lipid mixture of lecithin (8%), cholesterol (2%), propylene glycol (7% *v*/v), and curcumin (0.3, 1 and 3%) was dissolved at 60 °C. Then phosphate buffer saline (PBS) (10 mM, pH 7) was heated and added to this mixture under a fine stream by stirring at 700 rpm for 30 min. Finally, the suspension was sonicated for 2 min by a 20-kHz low-frequency ultrasonic probe sonicator to obtain the nanovesicles (Cur-PgL). The same procedure without curcumin was applied to prepare empty nanoliposomes. The free form of curcumin was prepared by dissolving curcumin (0.3%) in ethanol 30%.

The mean diameter of the nanoliposomal formulation was determined based on the dynamic light scattering (DLS) by a Zetasizer system manufactured by Malvern Instruments Ltd. (UK). The percentage of encapsulation efficiency (EE %) was determined based on the procedure described previously by Partoazar et al. [13] and Jaafari et al. [11]. Briefly, Cur-PgL suspension was centrifuged at 8000×*g* for 30 min at 4 °C. The sediment was washed three times with PBS. Then the recovered curcumin was heated to dry at 80 °C for 45 min and was dissolved completely in anhydrous methanol for analysis by a UV spectrophotometer (Cecil, UK) at 430 nm. The percent encapsulation efficiency (EE) of curcumin in formulation was calculated by the following equation:

$$EE\,\% = [(\text{total amount of added curcumin} - \text{amount of curcumin precipitate})/\text{total amount of added curcumin})] \times 100$$

Loading capacity (LC) of formulation was also calculated according to the following equation:

$$LC = \text{weight of curcumin in nanovesicles}/\text{weight of lipid}$$

The morphology of nanovesicles such as size distribution, shape, and particle aggregation was assessed by transmission electron microscopy (TEM) (Zeiss Em10C, Germany) using negative staining with 1% phosphotungstic acid. TEM measurements were performed on fresh and 60-day-old samples that were kept at room temperature.

Drug release assessment

The in vitro release profile of curcumin-loaded liposomes was determined by dialysis method which was modified from Zhao's method [8]. Curcumin nanovesicle suspension (2 ml) was placed in a dialysis bag (molecular weight cutoff of 10 kDa) and immersed in 100 ml phosphate buffer pH 7.4 containing 20% ethanol at a constant temperature of 37 °C and rotation speed at

100 rpm. At each predetermined time interval, 0.5 ml of sample was withdrawn and curcumin concentration was measured at 430 nm using UV spectrophotometer. To ensure that sustained release profile is not due to membrane, curcumin dispersion in the same concentration with Cur-PgL was studied under the same condition

Animals

Male Wistar rats (270–300 g) and New Zealand rabbits (2 kg) were provided from Tehran University of Medical Sciences, School of Medicine (Tehran, Iran). The animals were housed at room temperature (22–25 °C) under 12 h light/dark cycles with access to pellet food and water. They were acclimated to the room conditions for 1 week prior to the test day. The bedding materials for rats were autoclaved and replaced with new ones every day. The cages were also disinfected with povidone iodine solution 10% to increase the accuracy of microbiological assessments. The experimental procedures were approved (No.30288-158-03-94) by the Ethics Committee of Tehran University of Medical Sciences in agreement with the standards for the care and use of laboratory animals.

Experimental design

The biocompatibility assessments of different concentrations of curcumin Pg-liposomes were performed in burn injured rats by inducing a second degree burn model [14]. Then, the safety of the selected concentration was confirmed by using ISO standards irritation test on rabbits. According to the report guidelines, the preferred animal species is the albino rabbit due to its highly sensitive and light skin that makes it possible to observe even slightly skin irritation caused by a substance [15, 16]. Animals were anesthetized with ketamine (80 mg/kg, ip) and xylazine (10 mg/kg, ip). The dorsal skin was shaved and cleaned with ethanol 70%. Two sites on their dorsum surface were exposed to heated ironic cylindrical devices to burn an area of 2.5 cm^2 for 10 s. The devices were heated in boiling water (98 ± 1 °C) at least 20 min before application. The burn model was used in biocompatibility, wound healing, and antibacterial assessments. Also, neutral red uptake assay as a cytotoxicity test was used to evaluate the effect of different concentrations of curcumin Pg-liposomes on cell survival.

Four groups of six rats each were used in wound healing and bacteriological experiments as follows: liposome alone as the control group, silver sulfadiazine SSD cream group (1% *w*/*w*), Cur-PgL 0.3% group, and free curcumin 0.3% group. The burn sites were immediately treated topically after burn induction with 200 μl of given solutions with no dressing. The treatment was continued once a day for 18 days. Specimens were collected from the skin surface before daily treatment for bacteriological tests on days 4 and 8. The degree of healing was evaluated by histopathology test on skin biopsy specimens obtained from the anesthetized rats on day 8. Photography was followed until day 18 on the other burn wound of animals for wound surface measurements.

Biocompatibility assessments

Dose response

Primary wound assessment was done to select the effective dose of the Cur-PgL formulation on the burn skins. Injured rats with burn skins were randomly divided into three groups of three rats. Three concentrations of curcumin, 0.3, 1, and 3%, were applied daily in a volume of 200 μl per wound on burn sites for 5 days. The same procedure was done for the empty PgL as well. The wound reaction parameter including wound surface area as well as the exudate condition were observed on treated rats. Data were reported using the index as described in Table 1 for the burn wound assessment which were applied previously by Edraki et al. [14]. The burn wound with controlled exudates with less volume, clear or yellow in color, and good surface reduction compared to others was considered as an appropriate response.

Table 1 Keast's score for burn wound assessment as an index applied in clinical study [14]

Numerical score	Wound measurement[a]	Exudates volume	Exudate quality
0	0	None	Thin, watery, clear to yellow, usually odorless
1	<0.3	Exudate fully controlled	Thin, watery, pink to light red, usually odorless
2	0.3–0.6	Exudate controlled	Frank blood, bright red
3	0.7–1.0	Exudate uncontrolled	Thin, watery, white to cream, possibly foul odor
4	1.1–1.4	–	Thick, translucent to opaque, white to cream, possibly foul odor
5	1.5–1.8	–	–
6	1.9–2.2	–	–
7	2.3–2.5	–	–

[a] Wound measurement was estimated by area (cm^2) of wounds

Table 2 Wound evaluation (histopathologic aspect) according to Abramov's scoring system [18]

Grade	0	1	2	3
Epithelialization	None	Partial	Complete, but immature	Complete and mature
Angiogenesis	None	Up to 5 vessels	6–10 vessels	More than 10 vessel
Granulation tissue formation	None	Few fibroblasts	Moderate fibroblasts	Fully matured
Collagen deposition	None	Partial	Complete but irregular	Complete and regular
Inflammation	None	Mild	Moderate	Abundant

Histopathologic sections were studied under high-power field (HPF) microscopy

Cell culture and cytotoxicity test

Human dermal fibroblast (HDF) cells were obtained from the Iranian Biological Research Center. HDF cells were maintained in Dulbecco's modified Eagles Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), L-glutamine, penicillin, and streptomycin and were incubated at 37 °C in 5% CO_2. After reaching confluency, the cells were detached from the flask with Trypsin-EDTA. The cell suspension was centrifuged at 3000 rpm for 3 min and then resuspended in the growth medium for further study.

Neutral red assay was performed according to previously published protocol [17]. Briefly, treated cells were incubated for 3–4 h at 37 °C with 0.33% neutral red solution. The medium was eliminated, and the cells were washed with 0.1% $CaCl_2$ in 0.5% formaldehyde as fixative. The fixative agent was removed and then a solubilizing solution of 1% acetic acid in 50% ethanol was added in equal volume to the original volume of the culture medium. The cells were kept at room temperature for 10 min, and the absorbance of neutral red extract was measured at 550 nm using an ELISA microplate reader (Lisa Plus, Aspen Diagnostics, India). The cell survival in the control group was considered 100%, and the cell survival in the treated groups was compared with that in the control group. Cell morphology was assessed under an inverted microscope (Olympus IX70, Tokyo, Japan) at a final magnification of ×100 before and after treatment.

Skin reaction test

The irritative effects of the selected dose of Cur-PgL formulation was evaluated by the rabbit skin irritation test according to ISO 10993-10: 2010—part 10 [16]. The back of three healthy young adult albino rabbits was clipped of fur and divided into four sites with a 2.5 cm × 2.5 cm area. After 24 h, the application sites were exposed to 0.5 ml of the selected formulation and covered with bandage for at least 4 h. The appearance of each application site was observed after 1, 24, 48, and 72 h. Evaluation of skin reactions was scored on 0–4 grading scale in terms of erythema and edema. The scores were estimated by using the equation of Kojic et al. to describe the primary irritation index (PII) values as follows: 0—no irritation, less than 2—mildly irritating, 2 to 5—moderately irritating, and greater than 5—severely irritating [15].

Histopathology and wound measurement

Biopsy samples of skin burn wounds were fixed in formalin 10% and processed for paraffin embedding. Sections (4 mm) were deparaffinized and rehydrated for hematoxylin and eosin (H&E) staining. The skin tissues were interpreted in a random order under blindfold conditions by a pathologist and scored according to the scoring system of Abramov's method [18]. A score of 0–3 was considered to each section for inflammatory cells, collagen deposition, angiogenesis, granulation tissue formation, and epithelialization as explained in Table 2. In order to measure the wound surface area, the burn wounds were photographed on days 0, 8, and 18 by a digital camera and the wound area was measured by the ImageJ software. The percentage of wound reduction area was calculated using the following equation [19]:

$$\text{Wound reduction area}\% = [(\text{Wound area day 0} - \text{Wound area relative day})/\text{Wound area day 0}] \times 100.$$

Evaluation of bacterial infection in burn wound

In this study, spontaneous contamination of the wound was considered in antibacterial activity evaluation of Cur-PgL on burn rats [13, 20]. Briefly, identification and quantification of the bacteria were started with taking swabs from the burn wound of treated rats on days 4 and 8. The skin swabs were soaked and serially diluted in saline. Then, bacterial dilutions were cultured on brain heart infusion (BHI) agar plates in an incubator at 37 °C. After 48 h, the number of bacterial colonies was counted for quantification and reported as colony-forming unit (CFU)/ml. Also, the shape of the isolated bacteria was determined by the gram-staining technique. The bacteria were again cultured on BHI agar plates separately based on their shapes and the

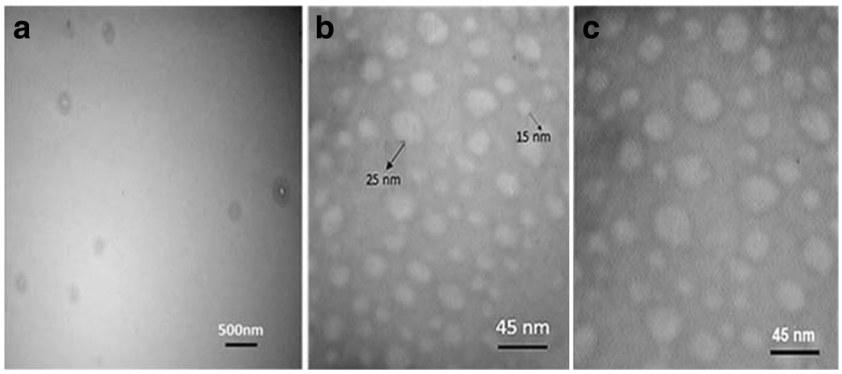


Fig. 1 TEM images of Cur-PgL nanovesicles (**a**) and fresh Cur-PgL suspension (**b**) with ×4000 and ×80,000 magnifications, respectively. **c** Cur-PgL nanovesicles image (×80,000) after 60 days storage at room temperature storage

identification of those bacteria was done by gram staining and biochemical assay including catalase, oxidase, bacitracin (0.04 U) resistance, arginine dehydrogenase, indole, motility tests, and other microbiology tests [21].

Statistical analysis

The differences in parametric data were assessed by one-way ANOVA followed by the Tukey's post hoc analysis. The data were expressed as mean ± SD. The histopathological scores were analyzed with the non-parametric Kruskal–Wallis test for overall significant differences among groups and reported as median. A P value <0.05 was considered significant.

Results and discussion

Characterization of Cur-PgL nanoliposomes

Vesicular nanocarriers have been extensively investigated as a dermal drug delivery system for different types of therapeutic agents, resulting in high entrapment efficiency, prolonged stability, and good efficiency [8, 9]. A particular study on PgL nanocarriers has shown several advantages over the other vesicular systems [8]. PgL nanocarriers have higher stability as well as better enhanced permeability and retention properties in the skin tissue compared to ethosomes and conventional liposomes [8]. It is believed that propylene glycol increases the elasticity of phospholipid bilayer in vesicles, thus enhancing the penetration of carrier-cargo into the deeper layers of the skin. Furthermore, it is proposed that the vesicles at sizes around 150 nm improve drug penetration and deposition into the skin layers [22]. As shown in Fig. 1a, b, the average size of nanoparticles in TEM fields was very close to the measured sizes (147 ± 6) with a good polydispersity index (0.41 ± 0.039) by DLS analysis (Table 3). As shown in the TEM images using negative staining (Fig. 1), the Cur-PgL nanocarriers were in spherical or oval shapes with a monodisperse vesicle population. There was no sign of nano-aggregation or drug crystallization in any fields.

The preliminary observation showed no average size change of Cur-PgL nanocarriers after 60 days of storage at room temperature (25 ± 2 °C) (Fig. 1c). The stability of the suspension could be related to the negatively charged vesicles (~−28 mv) caused by the incorporation of Pg excipient into the liposomal formulation (Table 3). Pg can facilitate the vesicle reformation from the lamellar phase and keep the curvature in closed vesicles as well [9]. In addition, the negative charge of Pg surface of the nanoliposomes produces an electrostatic repulsion force resulting in hindering the aggregation of nanoliposomes [8, 10]. The calculated encapsulation efficiency (85.3 ± 1.3) and the loading capacity (2.44 ± 0.18 μg/mg) for Cur-PgL 0.3% formulation are presented in Table 3. The results are in good agreement with the theory that esterified lipid bilayers possess an attractive loading capability for hydrophobic molecules such as curcumin [8].

In vitro release profile

In vitro curcumin release profile is an important index for evaluation of the quality and sustained release of curcumin-loaded vesicles. As shown in Fig. 2, more than 96% of curcumin was released from the free curcumin dispersion after 24 h through the dialysis membrane. On the other hand, after 7 and 24 h, only about 50 and 60% of loaded curcumin were released, respectively. The trend of the curve showed that

Table 3 Physiochemical characteristics of Cur-Pg nanoliposomes

Formulation	Average size (nm) by number	Polydispersity index	Zeta potential (mv)	EE %	LC(μg/mg)
Cur-PgL 0.3%	147 ± 6	0.41 ± 0.03 9	−28.53 ± 0.709	84.66 ± 2.4	2.44 ± 0.18

Data are reported as mean ± SD (n = 3)

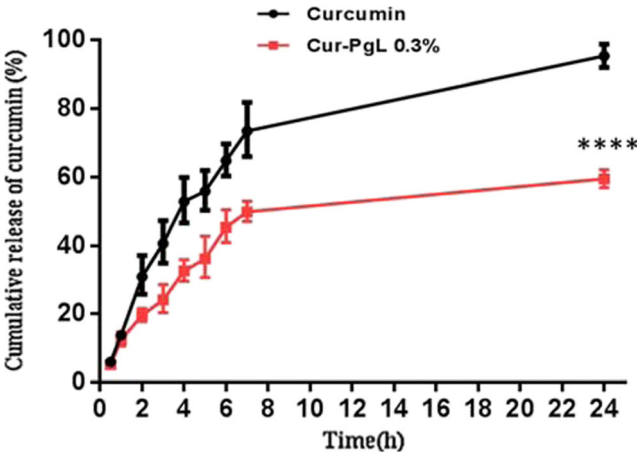


Fig. 2 In vitro cumulative release profiles of curcumin in nanoliposomal formulation versus the free form of curcumin (Mean ± SD, $n = 3$) through a semi-permeable membrane at 37 ± 0.5 °C; ****$P < 0.0001$

curcumin would continue to release from Cur-PgL nanovesicles within an extended period of time.

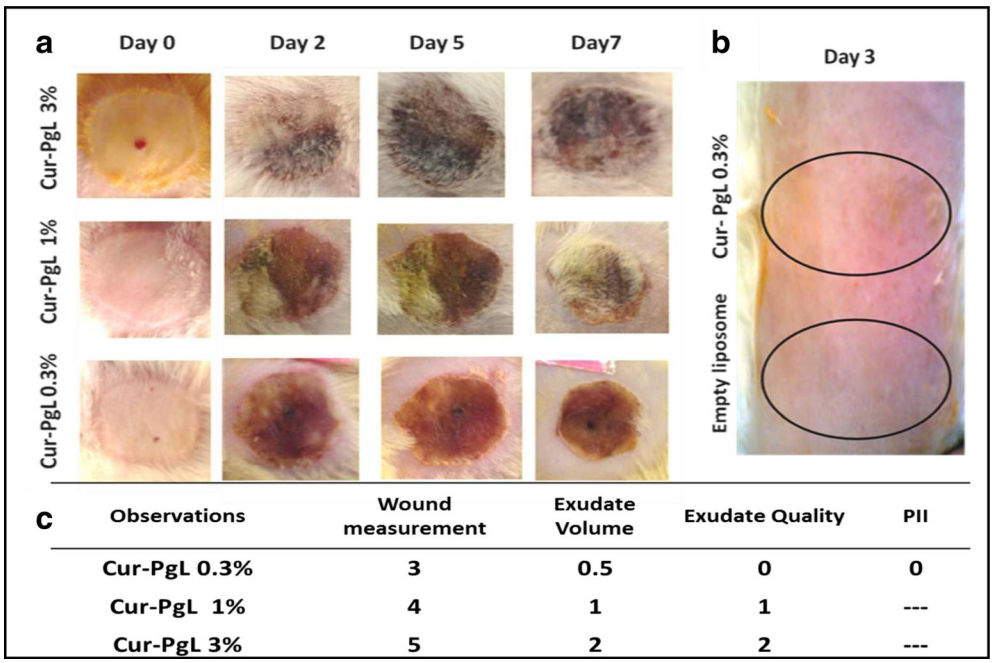
Biocompatibility and safety assessment of Cur-PgL

In this study, we used an approved formulation of natural ingredients with physical advantages described before. Liposomes are usually made of phospholipids, cholesterol, and water. The incorporation of activators such as ethanol and propylene glycol into the liposomes, as safe and nontoxic agents, is acceptable in pharmacy and drug formulations [10]. Propylene glycol is generally used in skin care, cosmetic, and pharmaceutical products and is approved by the Food and Drug Administration (FDA). Recent reports on PgL formulations have shown its biocompatibility and safety on both intact and impaired tissues [10, 11]. Ideally, a biocompatible material should be non-toxic with no side effects but with biologically beneficial effects at the same dose.

Curcumin is known as a natural drug with a vast food and industrial consumption. It might have side effects in high concentrations or at long-term dermal exposure due to its phenolic structure and vulnerability to sunshine [8, 23]. It is recommended that encapsulation of curcumin can overcome these deficits by reducing the dosage and sustained release in targeted delivery system [8].

As shown in Fig. 3a, c, the macroscopic appearance of wounds treated with Cur-PgL 0.3% was thin, light pink to yellow, with exudate fully controlled on day 7. On the other hand, the higher concentrations of curcumin had indifferent effects on wound repair that may be due to high deposition of curcumin in skin layers. The previous studies showed that the high concentrations of curcumin as nanogel or free form induced toxicity and apoptosis in cell cultures of human dermal fibroblasts [24]. The enhanced permeability and accumulation of the high concentration of curcumin by PgL in dermis layer where fibroblasts and other functional cells are localized might lead to cell cytotoxicity and thereby hinder the wound repair.

Our findings on HDF cell cytotoxicity test (Figs. 4 and 5) confirmed the significant toxicity ($P < 0.001$) of high concentrations of curcumin (1.5 and 3%) compared to the control group. Although, the impact of cytotoxicity of the vesicular curcumin 3% in terms of cell survival percentage with mean 4.4 ± 0.7 was less than the free form of curcumin with the same dose (11.1 ± 1.86). Lower toxicity of Cur-PgL compared with the free form of curcumin may be due to the sustained release of



c

Observations	Wound measurement	Exudate Volume	Exudate Quality	PII
Cur-PgL 0.3%	3	0.5	0	0
Cur-PgL 1%	4	1	1	---
Cur-PgL 3%	5	2	2	---

Fig. 3 **a** The macroscopic wound assessment after topical exposure to the different concentrations of Cur-PgL formulations. **b** Representative of rabbits' skin exposed to Cur-PgL 0.3% in skin reaction test (ISO 10993-10 standard). **c** Data from biocompatibility assessments ($n = 3$) show that Cur-PgL 0.3% acted as an effective formulation without irritation on the impaired and the intact skins

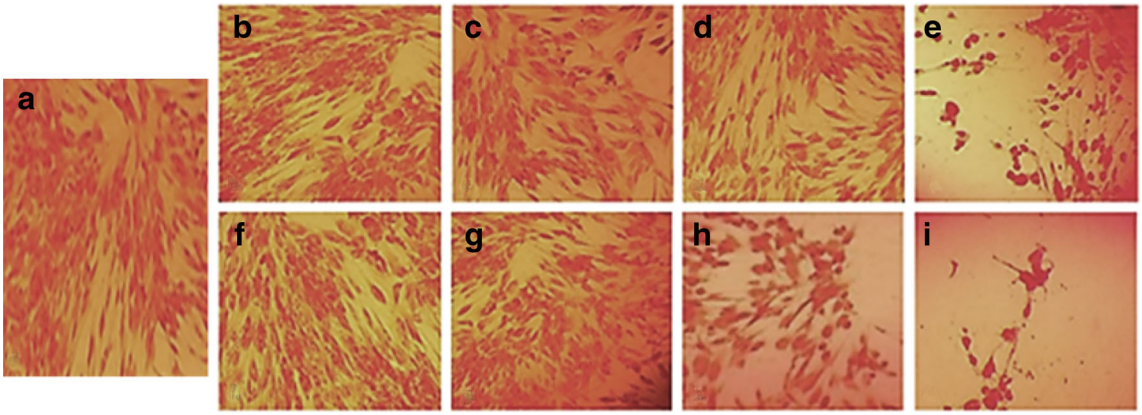


Fig. 4 Inverted micrograph of HDF cells in different drug concentrations. **a** Showing a spindle-shaped cell without any intervention. **b–e** HDF cells with curcumin loaded nanoliposomes 0.3, 0.7, 1.5, and 3%, respectively. **f–i** HDF cells exposed to free form of curcumin 0.3, 0.7, 1.5, and 3%, respectively. HDF cells were shrunken and rounded after exposure to high concentrations of both free form of curcumin and curcumin nanoformulation

the drug from the vesicular system as well as the low interaction of negatively esterified nanoliposomes with HDF cells [8]. The cell viability of about 100% in the treated HDF cells with Cur-PgL 0.3% (Fig. 5) indicates that this dose of curcumin has the minimum side effect.

The data of the primary irritation index of Cur-PgL 0.3% showed no irritability (PII = 0.0) as compared with the negative control group in rabbits (Fig. 3b). In conclusion, the results of all biocompatibility tests on the selected formulation showed that Cur-PgL 0.3% is safe and could be presented as a good candidate for burn therapy.

Antibacterial activity of Cur-PgL on burn wound

The study on microbial flora can help in recognition and control of environmental contamination and antibacterial susceptibility [25]. In this study, we designed a simple and reliable bacteriological evaluation of burn wounds that was previously described for wound healing assessment in rat [13, 20].

In this study, we detected *Staphylococcus aureus*, *S. epidermidis*, *Enterococcus faecalis*, *Bacillus cereus*, and *B. subtilis* as gram-positive bacteria and *Pseudomonas aeruginosa* as the gram-negative bacteria on the rats' wound. It is known that during the first few days following a burn injury, gram-positive bacteria of the endogenous skin flora or gram-negative bacteria of the gastrointestinal flora rapidly colonize on the burn wound surface [26]. *P. aeruginosa* and *Staphylococcus* species have a key role in bacterial infection of burn wounds [25, 26]. It is proposed that a chronic ulcer does not heal easily because of the presence of biofilms containing *P. aeruginosa* [27].

Our results in Table 4 showed that Cur-PgL 0.3% inhibited the bacteria growth isolated from the burn wounds significantly ($P < 0.001$) compared to the control group. There were no gram-positive bacterial colonies in the SSD group, but there were average numbers of colonial population of *P. aeruginosa* in both groups of SSD and Cur-PgL (data not shown). SSD showed stronger antibacterial activity compared to Cur-PgL 0.3% against the bacterial species of burn wounds. Although SSD is the most essential antibiotic for the burn infection therapy, it delays the healing process of deep partial-thickness burns in long-term application [28].

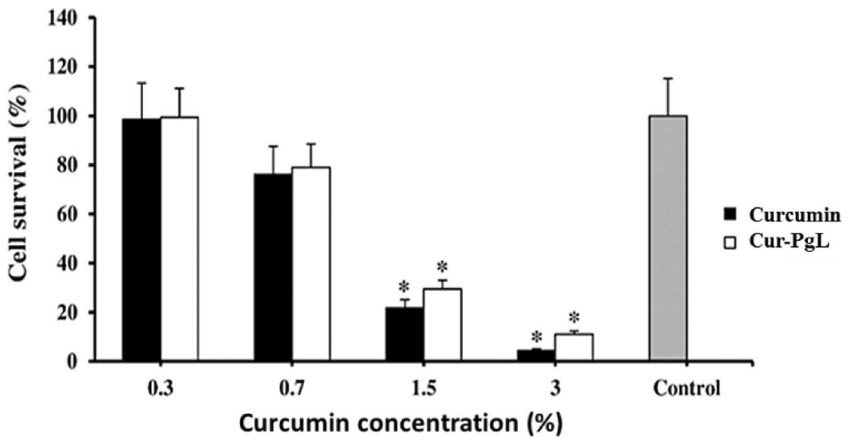


Fig. 5 Comparison of survival of HDF cells as mean ± SD with $n = 3$ after incubation with different concentrations of curcumin as a solvent of ethanol and liposomal formulation. The cell survival gradually decreased with the subsequent enhancement of curcumin concentrations *Significantly different from the control, with a $P < 0.001$

Table 4 Quantification of bacterial species related to the rat burn wounds as mean ± SD was estimated by CFU per milliliter (CFU/ml) at days 4 and 8. The percentage reduction of colonization (CFU/ml) for different groups is calculated in comparison with control group, ***; P<0.001

Experiments	Day 4	Day 8	Colonization reduction (%)
Control	$5.9 \pm 0.25 \times 10^6$	$8.2 \pm 0.15 \times 10^5$	–
Liposome	$5.3 \pm 0.13 \times 10^6$	$5.5 \pm 0.23 \times 10^5$	13.4%
Curcumin	$6.5 \pm 0.23 \times 10^5$	$6.3 \pm 0.31 \times 10^4$	89.3%***
SSD	$1.8 \pm 0.21 \times 10^4$	$2.9 \pm 0.3 \times 10^3$	99.4%***
Cur-PgL	$6.9 \pm 0.23 \times 10^4$	$8 \pm 0.13 \times 10^3$	98.8%***

Cur-PgL promote wound healing

The improvement of the wound healing by Cur-PgL might be attributed to the enhanced permeation of the vesicles with their cargoes into the skin layers which increases the local bioavailability of the drug [9]. Using the free form of curcumin by solving it in anhydrous ethanol caused precipitation and aggregation on the skin surface due to the rapid evaporation of the solvent which is not favorable phenomenon in dermal delivery [8].

Several studies on wound healing showed that curcumin increases the number of myofibroblasts, cellular proliferation, and collagen synthesis of wound tissues in animal models [4, 7]. In our study, as shown in Fig. 6, the percentage of wound surface was reduced significantly ($P < 0.001$) in the Cur-PgL group compared to the other groups after 18 days of treatment. A previous study showed that the considerable wound contraction of curcumin could be in relation to the increasing number of myofibroblasts and differentiation of fibroblast [4]. Our findings also showed that the low dose of curcumin in the liposomal formulation dramatically affected the main parameters of burn wound healing in a short period of time (Figs. 7 and 8). The median score of wound sections in the Cur-PgL group showed complete re-epithelialization in the epidermis ($P < 0.01$) and mature collagen bundles ($P < 0.001$) embedded at a regular granulation tissue ($P < 0.01$) with a continuous basement membrane as shown in Figs. 7 and 8. Collagens provide strength to the healing tissue upon appropriate synthesis, cross-linking, and alignment [4]. After wounding, fibroblasts and endothelial cells are the most important cell types in the reparative dermis which support the capillary growth, collagen development, and the formation of granulation tissue at the injury site [4]. The free form of curcumin accelerates the cellular proliferation and collagen synthesis in rats. Additionally, neovascularization which has the promoting effects on tissue regeneration [4] was observed significantly more in tissue sections of the Cur-PgL group (Fig. 7) compared to the other groups ($P < 0.05$). It is shown that curcumin can increase the neovascularization in wound healing by its effects on nitric oxide synthase [29]. It is suggested that inflammation has a key role in wound healing by infiltration of leucocytes which normally remove contaminating of microorganisms and cellular debris in the wound region [4]. On the other hand, the prolonged inflammation may cause further injury to other organs or delay wound repairing [30]. Thereby, there is a suggestion that both antibacterial and anti-inflammatory effects of the low dose of curcumin in the liposomal form could promote wound healing in burn injured rats.

Conclusion

In this study, Cur-PgL 0.3% was formulated in a fast and simple method without using any harmful chemicals and used for burn wound therapy in rats. Cur-PgL 0.3% nanocarriers

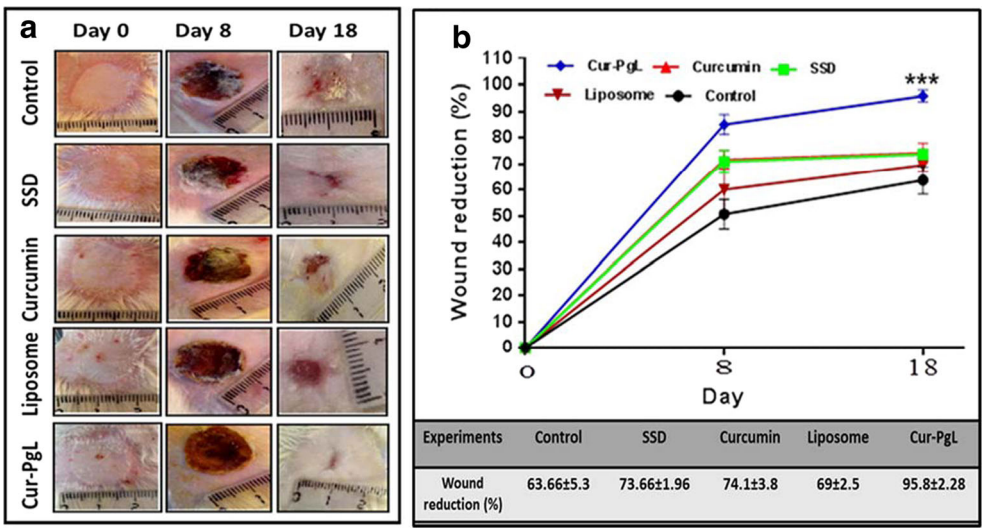


Fig. 6 **a** Representative photograph of wound appearances corresponding to the treated groups at different days. **b** The percentage of wound surface reduction was observed statistically (mean ± SD and $n = 6$) significant as ***$P < 0.001$ in rats treated with Cur-PgL 0.3% compared to the other groups after 18 days

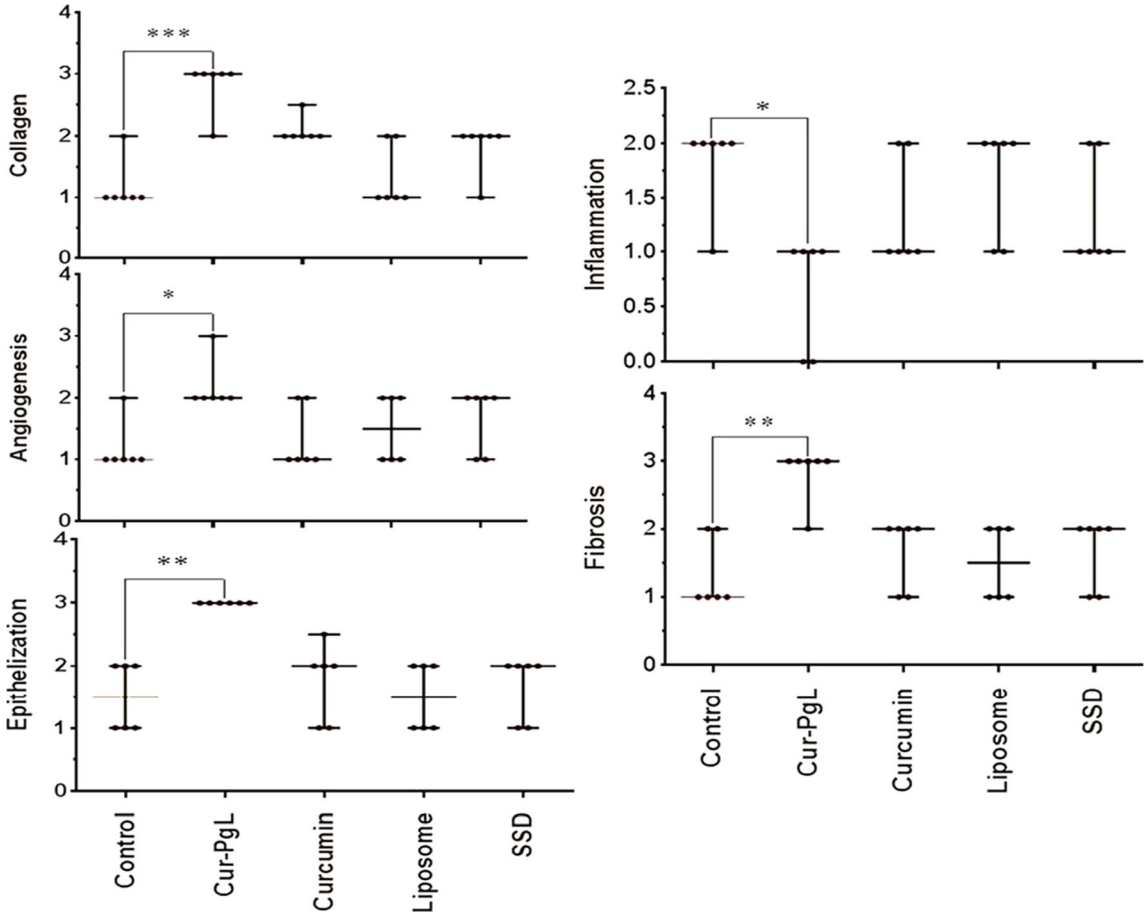


Fig. 7 Histopathology score analysis of the wound tissues (n = 6) as median ± SD expressed with error bars (min–max) after 8 days of treatment in rats subjected to the second degree burn induction. Significant difference values obtained from Cur-PgL 0.3% vs. control group were expressed by $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

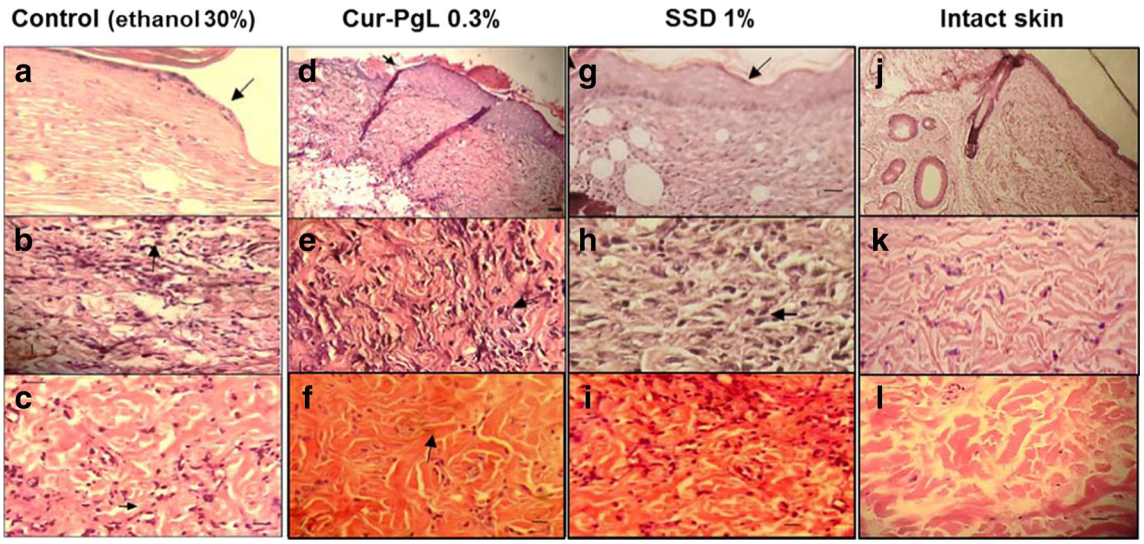


Fig. 8 Photography of histopathology with H&E staining related to sections as follows: Control sections with **a** partial or immature epithelialization, **b** few fibroblasts, and **c** partial and irregular collagen bundles. Cur-PgL group sections with **d** complete and mature re-epithelialization in epidermis, **e** matured granulation tissue, and **f** complete collagen bundles. **g** Complete but immature epithelialization, **h** moderate fibroblasts, **i** partial and irregular collagen bundles in sections are related to the SSD group. **j**–**l** Sections are related to a normal skin in untreated rat. Bar = 400 μm and Bar = 200 μm are indicated in collagen and epithelialization sections, respectively

were produced with good monodispersity and no aggregation even in long-term storage. Cur-PgL had considerable wound healing properties in the early stage and antibacterial activity on burn wounds similar to SSD. The biocompatibility of Cur-PgL was determined by the ISO10993–10 standard, the cell cytotoxicity test, and the wound assessment protocols that can be a key factor for using this formulation in translational medicine.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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