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Original Article

The impact of topical phenytoin loaded nanostructured lipid carriers in diabetic foot ulceration

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ABSTRACT

Objective: The aim of this study is to develop, and characterize nanostructured lipid carriers (NLCs) of phenytoin (PHT) in order to improve its entrapment efficiency and sustained release to improve the healing process.
Methods: Twenty-seven patients with neuropathic diabetic foot ulceration (DFU) were enrolled in this study. Patients were comparable regarding size, grading of ulcer and control of diabetes with no major deformity. All patients were managed by weekly sharp debridement if indicated and offloaded with cast shoes. They were equally divided into three groups: PHT-NLC-hydrogel (0.5%w/v), phenytoin hydrogel (0.5%w/v) and blank hydrogel groups. Changes in wound area were monitored over 2 months.
Results: Baseline wound area of PHT-NLC, PHT and blank hydrogels were 5.50 ± 3.66, 3.94 ± 1.86 and 5.36 ± 2.14 cm^2, respectively. Ulcers treated with PHT-NLC hydrogel showed smaller wound area compared to control groups ($\rho < 0.05$). The overall reduction in ulcer size were 95.82 ± 2.22% for PHT-NLC-hydrogel in comparison to 47.10 ± 4.23% and –34.91 ± 28.33% for PHT and blank-hydrogel ($\rho < 0.001$), respectively.
Conclusion: PHT-NLC hydrogel speeds up the healing process of the DFU without adverse effects when compared to the positive and negative control hydrogels. Moreover, the study can open a window for topical application of NLCs loaded with PHT in the treatment of numerous dermatological disorders that resist conventional treatment.
Key message: The delivery of drug molecules and their localization into the skin is the main purpose of the topical dosage forms. In this manuscript, the impact of topical phenytoin loaded nanostructured lipid carrier in improving wound healing in patients with neuropathic diabetic foot ulceration was investigated. Phenytoin loaded nanostructured lipid carrier dressing was found to be more effective than phenytoin hydrogel at the same concentration in healing of neuropathic diabetic foot ulcer.

1. Introduction

Diabetic foot ulceration (DFU) is a multifactorial disorder that includes several underlying factors. Petrova and Edmonds suggested that DFU results from factors extrinsic to the foot, such as infection, repeated trauma and ischaemia, as well as intrinsic factors that impair wound healing. Intrinsic factors are less well understood, they may include growth factors deficiency, changes in extracellular matrix components with excess proteases, reduced fibroblast activity, cellular abnormalities, nitric oxide abnormalities and angiogenesis deficiencies, in-addition to hyperglycemia. They suggested that therapies directed at correcting the intrinsic factors should be considered when treatments to correct extrinsic factors have failed [1]. The management of DFU requires offloading of the wound by using appropriate therapeutic footwear [2,3], daily saline or similar dressings to keep the moisture of the wound environment, debridement, when necessary, and optimal control of blood glucose [4].

Many studies involved different topical drugs to modulate internal wound environment such as povidone iodine solution, superoxidized solution, cadexomer iodine, hypochlorous acid, micronized collagen, acrylics, honey alginates [5].

The delivery of drug molecules and their localization into the skin is

Abbreviations: DFU, Diabetic foot ulceration; IRB, Institutional research board; NLC, Nanostructured lipid carrier; O/W, Oil in water; PEO, Polyethylene oxide; PHT, Phenytoin; SD, Standard deviation; w/w, weight/weight; w/v, weight/volume

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the main purpose of the topical dosage forms. Several considerations should be taken into account for developing an ideal topical dosage form in regards to its accumulation into the skin, its reservoir capacity, in addition to its low systemic absorption and toxicity [6]. There are experimental studies showing that topical application of phenytoin can enhance wound healing through stimulating collagen deposition, facilitating nerve regeneration, enhancing fibroblast proliferation, decreasing collagenase activity and wound exudate, glucocorticoid antagonism and antibacterial activity [7]. Numerous studies have been conducted on phenytoin to promote the healing of decubitus ulcers [8], epidermolysis bullosa [9], recalcitrant neuropathic DFU [10], venous stasis ulcers [11], traumatic wounds, burns and leprosy trophic ulcers [12]. Concerning these purposes, phenytoin is commercially available as a topical spray with high percentage of organic solvents (such as ethyl alcohol (about 70%) and/ or propylene glycol). Unfortunately, its use is restricted to once-daily application in order to avoid the adverse effects associated with its high contents of organic solvents such as skin dryness, redness, irritation, burning and allergic contact dermatitis. Hence, there is a need for new organic solvent-free topical formulations that can improve phenytoin wound healing ability with minimal or no adverse effects [13].

Recent attentions to nanostructured lipid carrier (NLC) researches have tended to focus on the topical (particularly dermal) application for pharmaceutical as well as cosmetic objectives [14]. They are prepared from physiological, biodegradable, non-irritating and non-toxic excipients (*e.g.* triglycerides, complex glycerides mixtures or waxes) [15,16]. Nanostructured lipid carriers are advantageous in comparison to other topical carriers (*e.g.* creams and emulsions) in several aspects due to sustaining the encapsulated drug release, drug targeting, being appropriate for incorporation lipophilic and hydrophilic drug molecules, protection of labile drug, bypass the use of organic solvents, large scale production, negligible skin irritability and ease of sterility [17,18]. In addition, they provide an occlusive effect because of the film formation on the skin surface and diminish transepidermal water loss [19] as well as high percutaneous absorption due to their high surface area and the penetration enhancing ability of their components [20].

The aim of this study was to develop and characterize a novel topical NLC for enhancing the entrapment efficiency and sustained release of phenytoin (0.5%w/v PHT-loaded NLC hydrogel), and study its impact on the healing of DFU in patients with no clinical evidence of ischaemia or infection.

2. Materials

Phenytoin: supplied from El-Nasr pharmaceutical Co. (Cairo, Egypt), Compritol 888 ATO (glyceryl behenate), Capryol 90 (propylene glycol monocaprylate) were received as gift samples from Gattefosse Co. (Lyon, France). Poloxamer 188 (Pluronic® F68) and sodium lauryl sulfate were purchased from Sigma–Aldrich Co. (Steinheim, Germany). Acetone was obtained from Piochem, Egypt. Carbomer® 934 (Carpobol® 934) was purchased from Techno Pharmchem Haryana, India. Triethanoalmine, propyl paraben, methyl paraben and propylene glycol were provided from Oxford Laboratory, India. All other chemicals and reagents were of analytical grade.

3. Methods

3.1. Preparation of PHT-loaded NLC

NLC was successfully prepared by hot homogenization and ultrasonication technique with slight modification to promote the lipophilic drug encapsulation into the nanoparticles [21]. It was prepared in the laboratory of Pharmaceutics Department- Faculty of Pharmacy- Mansoura University, Egypt. The solid lipid matrix (Compritol 888 ATO 1% w/w) was mixed with the liquid lipid (Capryol 90 1%w/w), maintained at 5 °C above its melting point to preclude any lipid memory effect and 0.05%w/w PHT was solubilized therein at 70 °C to form a drug-lipid mixture. Two milliliters of acetone were added under continuous stirring until the melt appeared clear. The aqueous phase was composed of 1%w/w non-ionic surfactant (Poloxamer 188) which was solubilized in distilled water and heated to the same temperature (70 °C) of the drug-lipid mixture. The hot aqueous phase was added to the lipid phase, and the mixture was homogenized using Heidolph homogenizer (Heidolph Instruments, Germany) at 20,000 rpm for 5 min to form an o/w emulsion. Then, this emulsion was sonicated (a probe-type sonicator, Sonics vibra cell, USA) at 45–50 W for 10 min in an ice bath to obtain NLC dispersion. Then, this dispersion was stirred for 2 h and centrifuged at 21,000 rpm using cooling centrifuge (Beckman Model J2-21 centrifuge, California, USA) for 2 h at 4 °C to separate the formed NLC. The nanoparticles residue was washed three times with distilled water and prepared for lyophilization (Freeze dryer, Gold-SIM, FD8-8 T, USA) by keeping at −8 °C overnight then, allowed to be lyophilized for three days [22]. All samples were prepared in triplicates.

3.2. Preparation of PHT-loaded NLC hydrogel

Carbomer was used as a viscosity-improving agent in order to obtain viscosity levels suitable for topical application. Briefly, propylene glycol solution (7%w/v) was mixed with the lyophilized PHT-loaded NLC (equivalent to 0.5 gm PHT). Carbomer 934 (1% w/v) was dispersed well in the NLC suspension. The mixture was neutralized to a pH value 6.5–7 with triethanolamine under mild stirring to induce polymer gelation. Methyl paraben and propyl paraben (ratio1:1) were used as preservatives in a concentration of 0.1%w/v. The prepared hydrogel was left overnight without stirring to get rid of any existing air bubbles then kept at 2–8 °C for the subsequent study. The same protocol was used for preparation of both blank and 0.5%w/v PHT-hydrogels as negative and positive controls, respectively.

3.3. Characterization of PHT-loaded NLC

3.3.1. Particle size analysis

Particle size and polydispersity index of the lyophilized NLC were analyzed by photon correlation spectroscopy using a Zetasizer23 (Nano ZS 90, Malvern, UK) after proper dilution by double distilled water.

3.3.2. Morphological investigation of PHT-loaded NLC

The shape and internal structure of the PHT-loaded NLC was determined with transmission electron microscopy (TEM, JEM-2000EX II Electron Microscope, JEOL, Tokyo, Japan) working at 80 kV. One drop of diluted sample was placed on the surface of carbon coated copper grid and allowed to dry for 2 min at room temperature for investigation [21].

3.3.3. In-vitro drug release study

In vitro release of PHT was evaluated by modified Franz diffusion cell [23] with slight modification using dialysis membrane of molecular cut off between 12,000–14,000 Da. Certain weight of the investigated gels (0.5 gm) was evenly spread on the diffusion cell surface (the donor compartment) whereas, the receptor compartment contained the dissolution medium (100 mL phosphate buffer pH 7.4 with 0.5%w/v sodium lauryl sulfate), which was continuously stirred at 50 rpm and maintained at 37 ± 0.5 °C. Aliquots (2 mL) of the dissolution media was withdrawn at predetermined time intervals up to 48 h, filtered through 0.2 µm Millipore filter and then, replenished with fresh medium each time to preserve the constant volume. In addition, *in vitro* dissolution of hydrogel containing free drug was investigated and was resembled as a blank. The percentages drug released were measured at λ_{max} 231 nm UV-spectrophotometer (JASCO, V-530, Japan) and different kinetic models [13] were determined to describe the release kinetics of the drug from the prepared hydrogels. Each measurement was

performed in triplicate.

3.4. Patient population

A prospective double blinded randomized controlled study was performed on 27 patients with diabetes and neuropathic foot ulceration. Consecutive patients with neuropathic DFU below the metatarsal heads (commonest site of DFU) attending Mansoura diabetic foot clinic from January 2017 to March 2017 were invited to take part in this study. Exclusion criteria included patients with chronic renal insufficiency, serious coagulopathies, immunosuppression, and those who have wounds that probed to bone on the initial visit or with a radiological evidence suggestive of osteomyelitis, as well as those with any clinical evidence of soft-tissue infection or ischaemia. Soft-tissue infection was identified when there was purulent secretion and/or two or more local signs of infection, such as erythema, warmth or tenderness. Ischaemia was identified with colored duplex for suspected cases with clinical signs or symptoms suggesting presence of ischaemia, also patients with ankle brachial pressure index lower than 0.9 were excluded. These exclusion criteria results in excluding only 2 patients, one with diabetic nephropathy, and another with soft tissue infection. All patients were managed by weekly sharp debridement and offloaded with identical cast shoes made by the same hands. The only dressing used was saline gauze, which is routinely used in our clinic due to lack of resources.

All patients were randomly assigned to one of the three groups by randomized block design based on age group, BMI and sex. The first group received a wound dressing with PHT-NLC hydrogel, the second group received a wound dressing with PHT-hydrogel, representing the positive control (PHT treatment), and the last was supplied with blank hydrogel, representing the negative control (no treatment). It was adjusted in such a way that the concentrations of PHT in the applied hydrogel and NLC were the same. The healing results were recorded by measuring the wound area then, calculating the wound healing percentage over 2 months. Also, the change in wound depth was monitored during the course of treatment using a sterile transport swab. To test for reliability of ulcer depth measurement, intraclass correlation coefficient was run on 10 patients for whom the depth was measured by two comparable investigators with a highly significant reliability ($p < 0.0005$). The wound was first cleansed with sterile isotonic saline, then the hydrogel was applied twice daily to cover the wound area and the wound was then covered with sterile gauze, this continued for a period of 8 weeks. All patients received one collapsible tube of about 25 gm of the hydrogel to make the dressing for one week. Patients were blind regarding type of the used dressing and they were instructed on guidelines for wound dressing and were asked to dress his/her ulcer daily and come back to the outpatient clinic every week for clinical evaluation and follow up. The study design was approved by the Institutional research board (IRB) of the Faculty of Medicine - Mansoura University, Egypt (IRB no. 16.12.57) including an informed consent from all patients as well as healthy individuals before sharing in this study.

3.4.1. Ulcer size measurement

The ulcer size was assessed using a transparent plastic paper placed on the ulcer and its outlines were drawn using a marker pen, then matched with the metric graph paper (expressed in cm^2) for estimating the wound area. Finally, the contraction of wound healing was monitored by calculating the wound healing percentage using the Walker formula [24]

$$\%\text{Wound area} = \frac{Wt}{Wi} \times 100$$

$$\%\text{Wound healing} = 100 - \%\text{Wound area}$$

Wt: the wound area at certain time point, Wi: the initial wound area.

3.4.2. Adverse events

The serum phenytoin levels were not monitored during this study but each patient was asked at each weekly visit about any treatment-related adverse events (e.g burning sensation) [[25]] that could have happened to them. In addition, the ulcers with the surrounding skin were carefully examined for any new abnormal changes (e.g hypertrophic granulation tissue formation [26], crusts or skin irritation [27]).

3.5. Statistical analysis

Statistical analysis of the clinical data was performed using one-way analysis of variance (ANOVA) followed by Tukey Kramer multiple comparison tests. The statistical analysis was calculated using Graphpad prism software version 5 (San Diego, CA, USA) at $\rho < 0.05$. All the results were interpreted as the mean values ± standard deviation (SD) at nine determinations ($n = 9$).

4. Results

4.1. Formulation and characterization of the prepared PHT-NLC

The mean particle size of PHT-NLC was uniform (178.2 ± 4.53 nm) with a narrow PDI distribution of less than 0.2. The PHT-NLC were almost spherical as shown in TEM image in Fig. 1.

The *in vitro* release profile of PHT from the lipid nanoparticles in phosphate buffer (pH 7.4) with 0.5%w/v sodium lauryl sulfate was presented in Fig. 2. A biphasic release is observed with burst release of PHT-NLC gel in the first 4 h followed by a steady state. PHT-gel has shown higher release rate than PHT-loaded NLC gel ($\rho < 0.001$). The release of PHT from the drug alone was very rapid, about 27.68 ± 4.39% of the drug was released within 4 h and 82.65 ± 4.71% drug release was observed within 48 h. On the contrary, the release of PHT from NLC was slow and approximately 14.39 ± 1.31% was released within 4 h followed by a slower and continuous release reaching 57.93 ± 2.84% in 48 h at pH 7.4.

The value of r^2 was found to be highest for the Higuchi kinetics (r^2 ranged between 0.8588–0.9545) as well as Korsmeyer n values of these formulations were found to be in the range of 0.53–0.49 for PHT and PHT-NLC hydrogels, respectively at pH 7.4. Whereas, the r^2 values of PHT- and PHT-NLC hydrogels were 0.6279, 0.6480 for first order and 0.8390, 0.8873 for second order, respectively.

4.2. Patient population

Twenty seven patients with diabetes (58 ± 8.7 years; 13 males), their mean body mass indices were (32 ± 2), mean duration of diabetes (in years) was 17.3 ± 6.8, and mean HbA1c (8.1 ± 1.2), all of them are on insulin therapy. The percentage of wound area (%) is an indicator of the contraction rate of the foot ulcer during the course of treatment. Table 1 illustrated that the percentage wound areas covered with PHT-NLC, PHT and blank hydrogels were changed to 26.61 ± 3.82, 71.89 ± 11.27 and 110.21 ± 17.36% after the 4^{th} week and by the end of the 8^{th} week, the percentage wound areas became 4.18 ± 2.22, 52.90 ± 4.23 and 134.91 ± 28.33% of their original size with PHT-NLC, PHT and blank hydrogels, respectively. The wound area was significantly reduced with PHT-NLC hydrogel compared to blank ($\rho < 0.001$) and PHT-hydrogels. All patients received the same standard care, even the casting was done by the same healthcare professionals.

In regards to the wound depth as described in Table 1, although there was a decrease in the wound depth with the nanoparticles *vs.* control groups, but this difference was not statistically significant ($\rho > 0.05$).

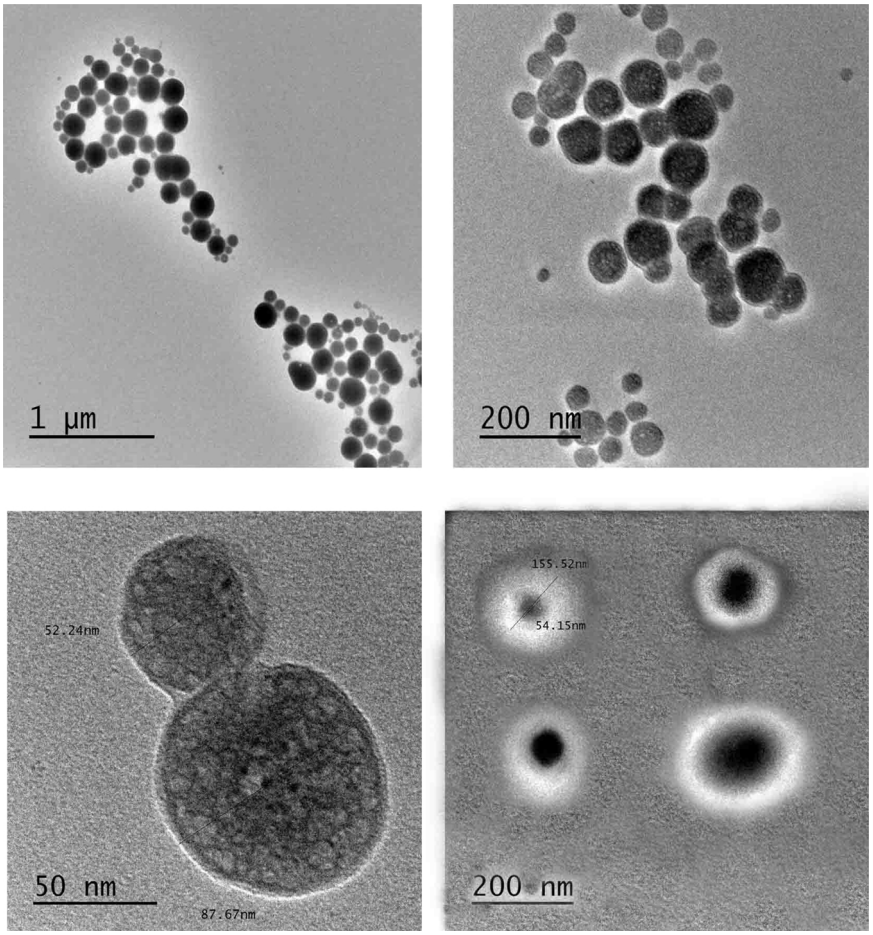

Fig. 1. Transmission electron microscope of phenytoin nanostructured lipid carrier formulation.

5. Discussion

In the present study, an economical, simple and reproducible method for the preparation of NLC, homogenization followed by ultrasonication was adopted at the above melting point of the lipid [28]. PHT- loaded NLC was composed of Compritol 888 ATO mixed with Capryol 90 (in ratio of 1:1) as core matrices and stabilized by Poloxamer (1%w/w). Surfactant plays a very crucial role in formation and stabilization of nanoparticles. Poloxamer 188 was used because of its safety and acceptability properties. Some problems were evident in systems stabilized through using low concentration of Poloxamer 188 surfactant (0.25% or 0.5% w/w). The particles were aggregated together within 2–3 days and then were converted into gel-like structure which may be due to characteristic crystalline behavior of the solid lipids and the disability of surfactant to cover newly formed surface of nanoparticles. The polypropylene oxide portion of the poloxamer anchors to the hydrophobic surface of particles, leaving the polyethylene oxide (PEO) chains to protrude into the surrounding aqueous media. Nevertheless, a low surface concentration allows a non-extended PEO chain conformation that enhances the formation of unstable particles. If the poloxamer surface concentration is increased, crowded surfactant surface favors a more extended PEO layer and acts as a steric stabilizer [29].

Sonication period did not significantly impact the particle size and ultrasonication for 10 min was appropriate to get particles of smaller sizes with narrow particle size distribution of less than 0.2 and consequently, reduced the production time and cost [21].

The Transmission electron microscope image indicated that the nanoparticles were almost spherical with smooth morphology which might be due to the combination of chemically heterogeneous lipids with heterogeneous surfactants aligned the rounded lipid nanoparticles formation [30]. Moreover, the majority of particles were distributed between 150–200 nm, which correlated well with the particle size analysis that was conducted using dynamic light scattering [31].

In vitro release study of the investigated gels is a crucial step in the development stages of new formulations and a routine quality-control test to confirm the final product uniformity.

In the *in vitro* release test, PHT-NLC gel showed an initial burst release in the first 4 h followed by sustained release. It may be ascribed to the diffusion of unentrapped drug in the first 4 h followed by diffusion from the NLC surface and thereafter from the core. Release data of PHT-NLC gel indicates that the investigated gels follow matrix diffusion based release kinetics and the release process was a coupling of diffusion and erosion mechanisms (anomalous non-Fickian transport, $0.45 \leq n \leq 0.89$). Thereby, the amount of drug released mainly was dependent on the matrix drug load [32,33,13].

From a therapeutic standpoint, both burst release and sustained release are recommended for topical products. Burst release can be useful to enhance the drug penetration and sustained releases prolong the residence time at the injury site as well as minimize the systemic absorption [34].

PHT-NLC application resulted in a greater reduction in wound areas and shorter healing time in comparison to the control groups throughout the study period. The results are clearly evident that the percentage wound healing with PHT- nanoparticles hydrogel was statistically more significant than that of blank ($\rho < 0.001$) and PHT-hydrogels ($\rho < 0.05$) (Table 1).

The patients had no adverse events or any abnormal changes in the

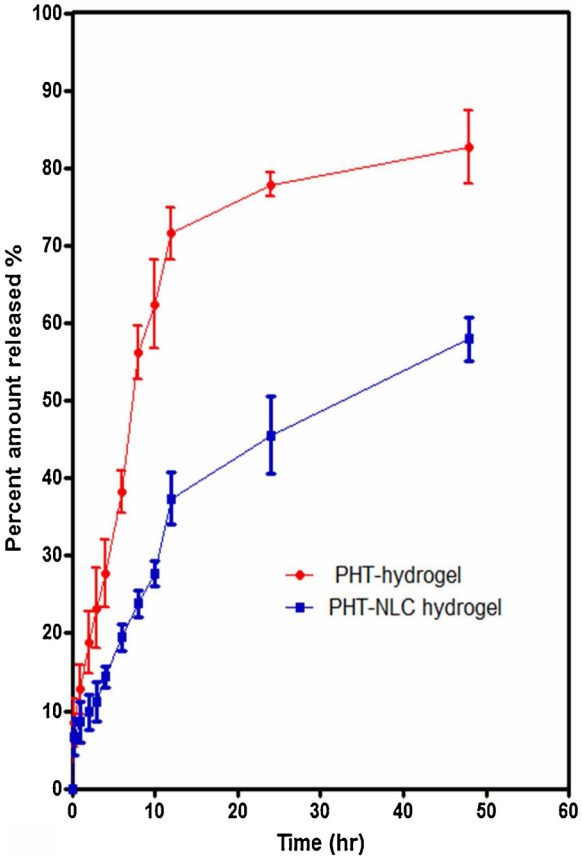

Fig. 2. *In vitro* release profile of phenytoin and phenytoin nanostructured lipid carrier hydrogels at pH 7.4 containing 0.5% w/v sodium lauryl sulfate.

wound area or the surrounding skin during our study. The hydrogel was constantly hydrating the wound to keep the wound moisture and avoid the formation of crusts or skin irritation that usually results from the direct application of phenytoin powder [27]. In addition, ulcers treated with PHT-nanoparticles were less exudative when compared to the negative control group, it could be attributed to the antibacterial activity of phenytoin when applied topically that may have resulted in a reduction of the bacterial load within the wound bed. Consequently, this may result in a decrease in the wound exudate that is one of the clinical markers of infection [35].

It is obvious from the photographic images (Figs. 3–5) that PHT-NLC hydrogel is more effective in wound closure when compared to the positive and negative controls throughout the duration of the study. There are many factors contributing to the increased skin delivery by NLC formula such as solubility enhancement, a large surface area due to small particle sizes, an occlusive effect and permeation enhancer [36]. The mechanism by which lipid composition in nanoparticles system enhances the skin penetration includes disruption of densely packed lipids that fill the extracellular spaces of the subcutaneous layer in contrast to conventional gel [33,14].

6. Conclusion

PHT-NLC-dressing is more effective than PHT-hydrogel at the same concentration in healing of DFU. This effect may be assigned to its small particle sizes with consequent increase in its solubility, in addition to their biphasic release pattern that is recommended for topical products. These promising results encourage large-scale trials for use of PHT-NLC in treatment of diabetic ulcers and other chronic wounds.

7. Limitation of the study

Although this study show promising results, it is a single-institutional study on a relatively small sized population sample, with relatively short follow up. Multicenter studies on large size sample of patients are needed with long follow up period to provide more evidence.

8. Brief summary

- Numerous studies have been conducted on phenytoin to promote the healing of ulcers
- Phenytoin is commercially available as a topical spray with high percentage of organic solvents that causes skin dryness, redness, irritation, burning and allergic contact dermatitis.
- Hence, there is a need for new organic solvent-free topical formulations that can improve phenytoin wound healing ability with minimal or no adverse effects.
- Nanostructured lipid carriers are advantageous in comparison to other topical carriers in several aspects with high percutaneous absorption due to their high surface area and negligible skin irritability.
- Our aim is to develop a novel topical NLC for enhancing the

Table 1
Follow up of the wound healing in patients receiving blank, 0.5%w/v PHT and PHT-NLC hydrogels over eight weeks.

Time (week)	Formula	Parameters[a]			
		Wound area (cm^2)	Wound area (%)	Wound depth (cm)	Wound healing (%)
Initial	Blank	5.36 ± 2.14	100 ± 0.00	0.73 ± 0.25	0.00 ± 0.00
	PHT	3.94 ± 1.86	100 ± 0.00	0.61 ± 0.33	0.00 ± 0.00
	NLC	5.50 ± 3.66	100 ± 0.00	0.73 ± 0.28	0.00 ± 0.00
Fourth	Blank	5.96 ± 2.71	110.21 ± 17.36	0.43 ± 0.21	−10.21 ± 17.36
	PHT	2.92 ± 1.61	71.89 ± 11.27[‡,b]	0.35 ± 0.15	28.11 ± 11.27[‡,b]
	NLC	1.50 ± 1.14	26.61 ± 3.82[‡,b,‡,c]	0.36 ± 0.16	73.39 ± 3.82[‡,b,‡,c]
Eighth	Blank	7.24 ± 3.65	134.91 ± 28.33	0.39 ± 0.14	−34.91 ± 28.33
	PHT	2.05 ± 0.89	52.90 ± 4.23[‡,b]	0.20 ± 0.09[†,b]	47.10 ± 4.23[‡,b]
	NLC	0.26 ± 0.22	4.18 ± 2.22[‡,b,‡,c]	0.09 ± 0.09[‡,b]	95.82 ± 2.22[‡,b,‡,c]

PHT: phenytoin; NLC: nanostructured lipid carrier.
*Indicates significant difference at $\rho < 0.05$.
[a] Mean ± SD, n = 9.
[b] Indicate blank-, PHT.
[c] Indicate blank- hydrogels.
† Indicate significant difference at $\rho < 0.01$.
‡ Indicate significant difference at $\rho < 0.001$.

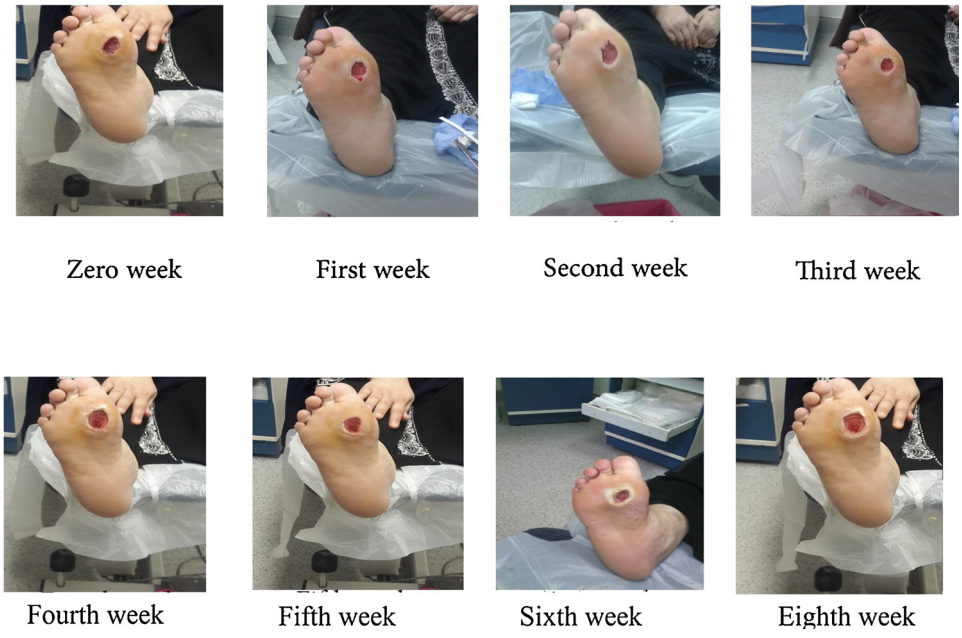

Fig. 3. The healing progress during eight weeks of blank-hydrogel.

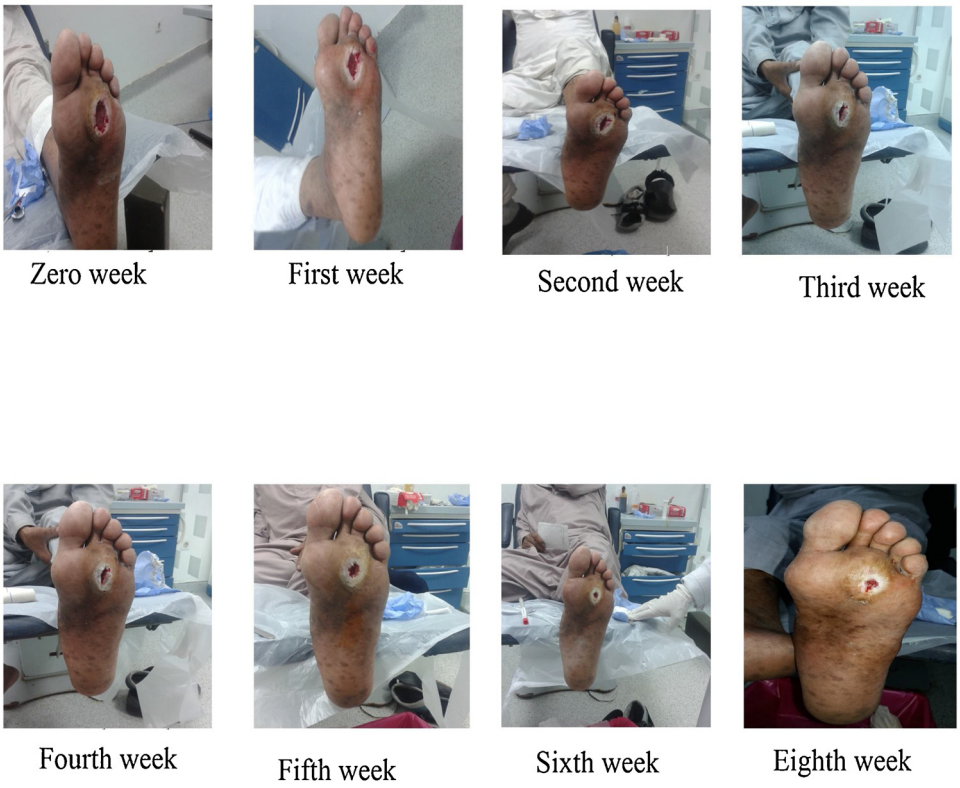

Fig. 4. The healing progress during eight weeks of 0.5%w/v phenytoin hydrogel.

entrapment efficiency and sustained release of phenytoin and study its impact on the healing of DFU in patients with no clinical evidence of ischaemia or infection.

- PHT-NLC-dressing is more effective than PHT-hydrogel at the same concentration in healing of DFU.

Authors' contributions

Motawea A. executed the idea, collected and assembled the data, did the data analysis and interpretation, and wrote the article. **Abd-El Gawad H.** helped to draft the manuscript and revise it critically with correction of typing and grammar errors. **Borg M.** conceived the study, participated in its design and coordination. **Motawea M.** carried out the *in vivo* part on diabetic foot patients, selected the cases, performed the statistical analysis and drafted the manuscript. **Tarshoby M.** participated in the design of the study and the statistical analysis. All authors read and approved the final manuscript.

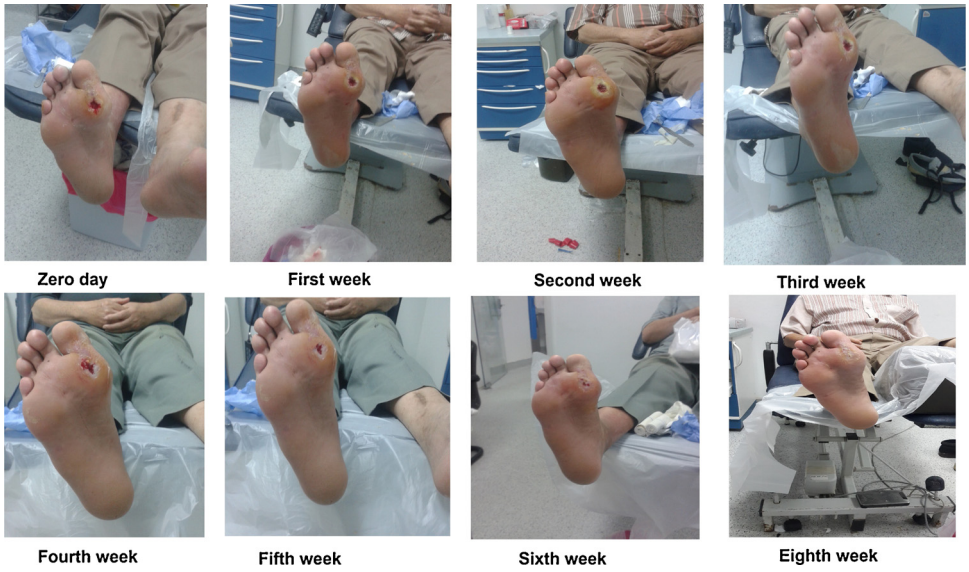

Fig. 5. The healing progress during eight weeks of 0.5%w/v phenytoin nanostructured lipid carrier hydrogel.

Disclosures

The manuscript is original, has not been submitted or published in whole or part elsewhere.

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Conflicts of interest

The authors report no conflicts of interest.

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