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ENHANCED WOUND HEALING ACTIVITY OF NANO LIPID CARRIER (NLC) GEL CONTAINING *Curcuma longa* AND *Syzygium polyanthum* EXTRACTS

T. R. Novianti[1], H. Kusriani[1,✉], and G. Jafar[2]
[1]Department of Biology Pharmacy, Bhakti Kencana University, Bandung-40164, Indonesia
[2]Department of Pharmacy Technology, Bhakti Kencana University, Bandung-40164, Indonesia
✉Corresponding author: herni.kusriani@bku.ac.id

ABSTRACT
The increasing global prevalence of diabetes mellitus, particularly its complications such as diabetic wounds, necessitates the development of safe, effective, and affordable therapeutic strategies. Natural products such as *Curcuma longa* and *Syzygium polyanthum* possess antidiabetic, antioxidant, and anti-inflammatory activities; however, their clinical application is limited by poor stability and bioavailability. This study aimed to develop a nanotechnology-based delivery system in the form of a Nano Lipid Carrier (NLC) gel incorporating combined herbal extracts to enhance topical delivery and wound healing efficacy. The extracts were prepared using 96% ethanol and characterised through standard phytochemical and physicochemical analyses. NLC formulations were optimised using Design Expert by varying lipid and surfactant compositions. The optimised NLC was evaluated for particle size, polydispersity index (PDI), zeta potential, and morphology, followed by incorporation into gel bases and assessment of physicochemical properties. In vitro diffusion and in vivo diabetic wound healing studies were conducted. The optimised NLC exhibited a particle size of 132 nm, PDI of 0.24, and zeta potential of −24.9 mV, indicating good stability. The Carbopol-based gel showed appropriate pH and viscosity. The NLC gel demonstrated sustained drug release, reaching approximately 68.6% cumulative diffusion at 24 hours, and significantly enhanced diabetic wound healing compared to conventional extract gel and control groups ($p < 0.05$). In conclusion, the developed NLC gel improves the stability, delivery, and therapeutic efficacy of *C.longa* and *S. polyanthum* extracts. This study supports Sustainable Development Goal 3 (Good Health and Well-being) by advancing accessible and effective therapies for chronic wound management.
Keywords: *Curcuma longa, Syzygium Polyanthum,* Nano Lipid Carrier, Diabetic Wound Healing.
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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterised by chronic hyperglycemia accompanied by disturbances in carbohydrate, protein, and lipid metabolism resulting from impaired insulin secretion or action.[1] The global burden of diabetes continues to increase, and Indonesia ranks among the countries with the highest number of cases. According to the International Diabetes Federation (IDF), the prevalence of diabetes in Indonesia reached 10.8% (approximately 19.5 million cases) in 2021 and is projected to rise to 28.6 million cases by 2045.[2] Although various pharmacological therapies are available to control blood glucose levels and prevent acute and chronic complications[2,3], diabetic foot ulcer (DFU) remains one of the most severe and challenging long-term complications of diabetes. DFU is the leading cause of non-traumatic lower-limb amputation worldwide, with a global prevalence of approximately 6.3% among patients with diabetes.[4] The condition is associated with delayed wound healing, high risk of infection, peripheral neuropathy, and peripheral vascular disease, which significantly impair patients' physical, psychological, social, and economic well-being. Uncontrolled diabetic wounds may result in reduced mobility, depression, anxiety, and substantial healthcare costs. These challenges highlight the urgent need for more effective and innovative therapeutic strategies for managing chronic diabetic wounds.[5] Among various medicinal plants, *C.longa* and *S. polyanthum* have been widely used in traditional medicine and scientifically reported to exhibit antioxidant, anti-inflammatory, antibacterial, and antidiabetic activities.[6,7,8] Curcumin, the major bioactive compound in turmeric, plays an important role in modulating oxidative stress, inflammation, and glucose metabolism[6,7], while Syzygium leaves

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contain flavonoids and other phenolic compounds that contribute to antimicrobial and wound-healing activities.[8] However, the clinical application of these herbal extracts in topical formulations is limited by poor stability, low solubility, and insufficient skin penetration when formulated conventionally. Nanostructured Lipid Carriers (NLCs) represent a second-generation lipid nanoparticle system designed to overcome these limitations. By incorporating liquid lipids into a solid lipid matrix, NLCs create a less-ordered internal structure that enhances drug loading capacity, improves stability, and enables controlled drug release.[9,10] Furthermore, embedding NLCs into a gel dosage form offers additional advantages, including improved spreadability, cooling sensation, non-greasy texture, and better patient compliance, making it particularly suitable for chronic wound management.[11] In addition, nanotechnology-based systems have demonstrated promising potential in accelerating wound healing processes.[11,12] Therefore, this study aims to develop and optimise an NLC-based gel containing combined extracts of *C.longa* and *S. polyanthum* as a potential topical therapy for diabetic wound healing. This research is expected to provide a scientific basis for improving the therapeutic potential and clinical applicability of herbal-based NLC gel formulations in the management of diabetic wounds. However, previous studies have primarily focused on single herbal extracts, with limited investigation into combined herbal formulations in NLC systems.[12] Therefore, this research is expected to provide a scientific basis for enhancing the therapeutic potential and clinical applicability of herbal-based NLC gel formulations in the management of diabetic wounds. By targeting the challenges of diabetic wound care, this study supports Sustainable Development Goal 3 (Good Health and Well-being) through the development of accessible healthcare solutions aimed at reducing the chronic disease burden.[13,14]

EXPERIMENTAL

Materials

Ethanol (96%) was used for extraction. Precirol® ATO 5 and oleic acid functioned as the solid and liquid lipids in the NLC system, while Tween 80 and Span 80 served as surfactants. The gel was formulated using Carbopol 940, triethanolamine, propylene glycol, and glycerin. In vitro release was evaluated using Franz diffusion cells with synthetic membranes and phosphate-buffered saline (PBS, pH 7.4). Male Wistar rats (200–250 g) were used as the in vivo diabetic wound model. Diabetes was induced by alloxan, and wound creation was performed under ketamine–xylazine anesthesia. All reagents were of analytical grade and used without further purification.

Plant Material

C.longa and *S. polyanthum* was obtained from Manoko Plantation, Lembang, Indonesia. Botanical identification was performed at the Plant Taxonomy Laboratory, Universitas Padjadjaran (No.551/LBM/IT/VI/2025 and No.559/LBM/IT/VI/2025). The plant was washed, dried at 40°C, powdered, and stored in airtight containers.

Extraction

Maceration was performed using 96% ethanol (1:10 w/v) for 3 × 24 hours (10). The filtrates were combined and concentrated using a rotary evaporator at 50°C. Extract yield respectivelly 19.8% (*C. longa*) and 17.6% (*S. polyanthum)*

Standardisation of Simplicia and Extract

Standardisation of *C.longa* and *S. polyanthum* simplicia was performed according to the Indonesian Herbal Pharmacopoeia.[14]

Preparation of NLC

The *C.longa* and *S. polyanthum* extract, loaded nanostructured lipid carriers (NLC) were prepared using a hot homogenization probe sonication method, which is widely employed to obtain nano-sized lipid dispersions with enhanced stability (8-10). The formulation consisted of a solid lipid (Precirol ATO 5), liquid lipid, surfactant, and co-surfactant. Initially, the solid lipid was melted and mixed with the extract under continuous stirring using a magnetic stirrer for 15 minutes to ensure uniform dispersion of the active compounds. In parallel, the solid and liquid lipid phases were heated simultaneously to maintain a uniform temperature. The hot lipid phase was then subjected to high-shear homogenization at 10,000 rpm

to form a coarse emulsion. Subsequently, size reduction was achieved by probe sonication at 60% amplitude and 70°C, resulting in the formation of NLC with a stable internal lipid matrix suitable for encapsulating the active compound.[8-12]

Characterisation of NLC

Physicochemical characterisation of the NLC system included measurement of particle size, polydispersity index (PDI), and zeta potential using dynamic light scattering (Zetasizer Nano, version 7.2). Encapsulation efficiency (EE) was determined to quantify extract entrapment. Morphology was analysed by transmission electron microscopy (TEM) at 120 kV. Stability was assessed on day 0 and day 30 by re-evaluating particle size, PDI, and zeta potential.[8-10]

Gel Formulation

The gel formulation containing *C.longa* and *S. polyanthum* extract loaded NLC was developed and optimised using Design Expert® version 4.3 through a factorial design approach to determine the optimal polymer composition and rheological characteristics. Carbopol 934 and hydroxypropyl methylcellulose (HPMC) were used as gelling agents within a concentration range of 1–2.5%. Glycerin (5%), propylene glycol (10%), and DMDM hydantoin (0.3%) were incorporated as a humectant, penetration enhancer, and preservative, respectively.[5,16] Triethanolamine (TEA) was added as a neutralising agent to adjust pH and facilitate gel formation in the Carbopol-based system. The incorporation of NLC into the gel base was performed under continuous stirring to obtain a homogeneous formulation.[17,18]

In Vitro Release (Franz diffusion)

The in vitro release and permeation of the *C.longa* and *S. polyanthum* NLC gel were evaluated using vertical Franz diffusion cells according to dermatopharmacokinetic guidelines.[16] A hydrated cellulose acetate membrane was mounted between the donor and receptor compartments, and the receptor chamber was filled with phosphate-buffered saline (PBS, pH 7.4), maintained at 32 ± 0.5 °C and continuously stirred.[16] A fixed amount of gel was placed in the donor compartment, and samples were withdrawn from the receptor compartment at predetermined intervals (0.5, 1, 2, 4, 6, 8, and 24 h), with immediate replacement by fresh medium. The released active compound was quantified using a validated UV spectrophotometric method. Cumulative release (% and $\mu g/cm^2$), Q24, permeation flux (J), permeability coefficient (Kp), and T50% were calculated; flux was obtained from the linear portion of the release curve under steady-state conditions, and Kp was determined as the ratio of J to the initial donor concentration.[8,19,20]

In vivo Diabetic Wound Healing

The in vivo wound-healing activity was evaluated using a diabetic wound model in male rats according to previously reported methods.[4,10] Animals were acclimatised for 7 days under controlled conditions (24 ± 2 °C; 12 h light/dark cycle), and baseline body weights were recorded. Diabetes was induced in Groups 2–5 by intraperitoneal injection of alloxan (150 mg/kg BW), and hyperglycemia was confirmed when blood glucose levels exceeded 200 mg/dL. Under anaesthesia, a full-thickness circular excisional wound (~1 cm diameter) was created on the dorsal area using a sterile scalpel, followed by rinsing with sterile saline. Treatments were applied once daily according to group allocation: negative control (base gel), standard (Sanoskin gel), crude extract gel, and extract-loaded NLC gel. Wound healing was evaluated on days 0-14 by measuring wound area with a digital calliper, and the percentage of wound closure was calculated relative to the initial wound size. Photographic documentation was used to support macroscopic observation.[11,12]

Statistical Analysis

All quantitative data were expressed as mean±standard deviation. Statistical analysis was performed to evaluate differences among treatment groups for physicochemical parameters, in vitro release profiles, and in vivo wound-healing outcomes. One-way analysis of variance (ANOVA) was applied to determine overall group differences, followed by Tukey's post hoc test for pairwise comparisons. A significance threshold of $p < 0.05$ was considered statistically meaningful.

RESULTS AND DISCUSSION

The standardization of *C.longa* and *S. polyanthum* leaf simplicia was performed in accordance with the Indonesian Herbal Pharmacopoeia.[12] All evaluated parameters, loss on drying, total ash, acid-insoluble ash, and extractive values, complied with the specified requirements. Loss on drying for both materials was below 10%, indicating acceptable moisture content. The total ash and acid-insoluble ash values of turmeric rhizome (6% and 0.5%) and Syzygium leaf (1.5% and 1%) were within permissible limits, suggesting minimal inorganic contamination. In addition, both water- and ethanol-soluble extractive values exceeded the minimum standards, reflecting a substantial presence of extractable bioactive constituents. These findings confirm that both simplicia met the pharmacopoeia quality criteria and were suitable for subsequent formulation development.[15]

NLC Characterization

The physicochemical characteristics of *C.longa* and *S. polyanthum* NLC formulations at initial preparation (T0) and after 30 days of storage (T30) are presented in Table-1. All formulations exhibited nanoscale particle sizes (104–288 nm) with relatively low PDI values (<0.3), indicating a homogeneous particle distribution.[8,17] Among the formulations, Formula 7 showed the most favourable characteristics, with a particle size of 125.47 ± 0.38 nm at T0 and 132.03 ± 2.44 nm at T30, indicating minimal change during storage. The PDI remained below 0.3, confirming a narrow size distribution. Zeta potential values ranged from −21.30 ± 1.14 mV to −24.90 ± 0.56 mV, suggesting adequate colloidal stability. Statistical analysis revealed that changes in particle size and PDI between T0 and T30 were not significant ($p > 0.05$), indicating good physical stability over time. The superior stability of Formula 7 can be attributed to its optimised lipid–surfactant composition. The use of Precirol as a solid lipid combined with Myritol as a liquid lipid likely contributed to the formation of a less-ordered lipid matrix, which enhances drug accommodation and reduces the risk of particle aggregation.[8,9] In addition, the presence of Cremophor as a non-ionic surfactant provides steric stabilisation, which is known to be effective in maintaining nanoparticle dispersion even at relatively low zeta potential values.[18] This explains why Formula 7 remained stable despite having zeta potential values below the conventional ±30 mV threshold. Similar findings have been reported, where non-ionic surfactants improve nanoparticle stability through steric hindrance rather than electrostatic repulsion.[8,17]

Table-1: Physicochemical Characteristics of NLC Extract at T0 and T30

Formula	T0 (day)			T30 (day)		
	Z-Ave (nm)	PDI	ZP (mV)	Z-Ave (nm)	PDI	ZP (mV)
1	195.20 ± 1.54	0.28 ± 0.01	-53.80 ± 0.62	189.60 ± 4.37	0.31 ± 0.04	-45.23 ± 1.61
2	235.87 ± 2.15	0.31 ± 0.12	-48.87 ± 0.95	163.87 ± 2.74	0.28 ± 0.02	-48.23 ± 0.84
3	214.70 ± 3.32	0.23 ± 0.02	-52.13 ± 1.65	206.07 ± 6.18	0.23 ± 0.02	-48.37 ± 0.84
4	277.90 ± 5.01	0.30 ± 0.13	-51.17 ± 1.18	208.00 ± 1.51	0.24 ± 0.02	-52.73 ± 0.12
5	261.93 ± 5.02	0.36 ± 0.03	-53.07 ± 1.87	201.93 ± 1.12	0.23 ± 0.03	-50.60 ± 0.98
6	104.50 ± 2.49	0.26 ± 0.02	-17.70 ± 0.95	119.07 ± 2.40	0.27 ± 0.02	-21.93 ± 0.57
7	125.47 ± 0.38	0.21 ± 0.02	-21.30 ± 1.14	132.03 ± 2.44	0.24 ± 0.02	-24.90 ± 0.56
8	151.97 ± 1.88	0.19 ± 0.02	-23.87 ± 1.10	186.43 ± 4.03	0.26 ± 0.05	-29.63 ± 1.10
9	183.57 ± 0.76	0.14 ± 0.04	-22.50 ± 1.20	197.60 ± 5.05	0.21 ± 0.05	-29.33 ± 0.64
10	288.10 ± 4.85	0.22 ± 0.20	-20.63 ± 1.18	267.63 ± 2.05	0.19 ± 0.02	-31.50 ± 0.87

Transmission Electron Microscopy (TEM) Analysis

Transmission Electron Microscopy (TEM) was employed to evaluate the morphology, particle size, and dispersion of both blank and extract-loaded NLC systems. The TEM micrographs (Fig.-1) revealed predominantly spherical nanoparticles, indicating successful formation of lipid carriers.[8] Such morphology is favourable for topical delivery, as it enhances surface contact and spreading on the skin.[8,10] A clear difference in particle size and distribution was observed between the two systems. The blank NLC exhibited particle sizes ranging from 81 to 94 nm with slight agglomeration, which is typical for lipid-based nanoparticles. In contrast, the extract-loaded NLC showed smaller particle sizes (48–58 nm) and more uniform dispersion. This size reduction is likely attributed to interactions between the lipid matrix

and bioactive compounds, which improve interfacial stabilisation and emulsification efficiency.[8,21] Furthermore, the extract-loaded NLC also demonstrated better homogeneity, indicated by fewer agglomerates and more evenly distributed nanoparticles. Although slight aggregation was observed in both formulations, it was less pronounced in the extract-loaded system. These results suggest that constituents of *C.longa* and *S. polyanthum* contribute to improved stabilisation of the NLC system. Overall, extract incorporation not only provides therapeutic potential but also enhances structural uniformity and stability of the formulation.[8,21]

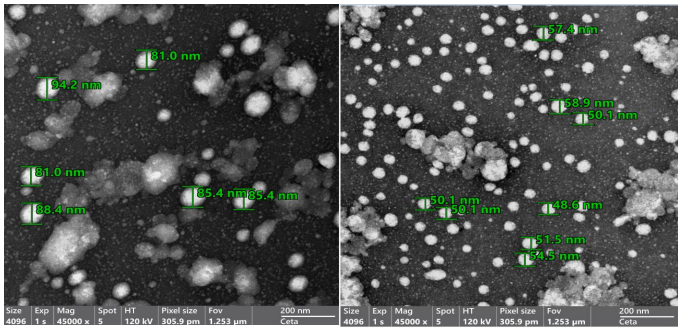

Fig.-1: TEM Micrographs of (a) Blank NLC and (b) Extract–Loaded NLC

Formulation and Evaluation of NLC Gel

The *C.longa* and *S. polyanthum* extract-loaded NLC gel was evaluated to determine the effect of polymer interactions on its physicochemical properties, including homogeneity, viscosity, spreadability, pH, and physical stability.[7,10] The optimised formulation produced a stable gel matrix with uniform nanoparticle dispersion and no syneresis, confirming its suitability for topical diabetic wound applications. The NLC gels met standard topical formulation requirements. The viscosity (2000–4000 cps) ensured ease of application and adequate skin adhesion, supporting drug retention at the wound site and prolonged therapeutic action.[11,12] The pH remained within the acceptable dermal range (4.5–6.5), indicating good skin compatibility. No phase separation or changes in colour and texture were observed, confirming physical stability. These findings indicate that the selected polymer system effectively maintains formulation stability while supporting drug retention. Consistent with previous studies, NLC-based gels enhance skin penetration and localised drug delivery due to their nanoscale size and lipid matrix, which facilitate interaction with the stratum corneum.[9,17]

***In vitro* Release Profile**

The in vitro diffusion study was conducted to evaluate the release behaviour of active compounds from the prepared formulations. As shown in Fig.-2, the cumulative release profiles differed among the three formulations: the extract gel, NLC dispersion, and NLC-based gel. The extract gel showed a pronounced initial burst release, indicating rapid diffusion of unencapsulated active compounds from the gel surface. In contrast, the NLC-based gel exhibited a slower initial release, reflecting effective encapsulation within the lipid matrix and reduced burst effect. Notably, the NLC-based gel achieved the highest cumulative release, reaching nearly 10% at 480 minutes, compared to the NLC dispersion (~8%) and extract gel (~7%). This suggests that incorporating NLC into the gel system enables both controlled and enhanced drug release. The sustained release profile indicates a reservoir effect, where the lipid matrix gradually releases the encapsulated compounds, prolonging drug availability. Additionally, improved release may be attributed to enhanced interaction between lipid nanoparticles and the membrane, facilitating better permeation, consistent with previous reports.[8,17,19] Statistical analysis using one-way ANOVA followed by Tukey's post hoc test revealed significant differences among the three formulations at all timepoints ($p < 0.05$). The NLC-based gel showed significantly higher cumulative release at later time points while maintaining a more controlled initial release compared to the other formulations.

***In vivo* Wound Healing**

An in vivo wound healing study was conducted to evaluate the therapeutic efficacy of the extract-loaded NLC gel under diabetic conditions. Figure-3 illustrates the macroscopic changes in wound appearance

from day 0 (T0) to day 10 (T10) across all experimental groups. At the initial stage (T0), all groups exhibited similar wound characteristics, indicating a uniform baseline condition before treatment. As the healing process progressed, differences among treatment groups became increasingly evident.

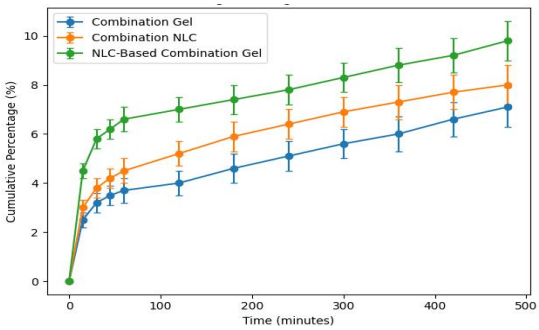

Fig.-2: Cumulative Release Percentage of the Drug Diffused
*Data are presented as mean ± SD (n = 3)

The wound closure percentages for all treatment groups are presented in Table-2. Diabetic wounds are known to heal slowly due to disrupted angiogenesis, prolonged inflammatory responses, and diminished extracellular matrix remodeling.[11,12] All groups exhibited a gradual increase in wound closure over time; however, the healing rate differed significantly among treatments. The NLC gel showed the fastest and most consistent response, achieving 43.89 ± 2.43% closure by day 4, markedly higher than the extract gel (25.91 ± 0.45%) and the standard treatment. Complete wound closure (100%) was observed in the NLC gel group by day 7, comparable to the positive control, whereas the extract gel required up to day 8, and the negative control showed minimal healing.[22,23] These results clearly demonstrate the superior therapeutic efficacy of the NLC-based formulation over both non-encapsulated and conventional systems. The enhanced healing performance can be attributed to improved drug delivery and retention at the wound site. The nanoscale size facilitates deeper skin penetration, while the lipid matrix enables sustained release, maintaining effective drug concentrations over time. In addition, bioactive compounds from *Curcuma longa* and *Syzygium polyanthum* likely contribute synergistically through antioxidant and anti-inflammatory mechanisms that promote tissue regeneration. These findings are consistent with previous studies demonstrating that lipid-based nanocarriers accelerate wound contraction and epithelialization via controlled drug delivery and enhanced tissue interaction.[6,10,13] Statistical analysis using one-way ANOVA followed by Tukey's post hoc test confirmed significant differences among groups at several time points ($p < 0.05$), particularly between the NLC gel and the negative control, as well as between the NLC gel and non-nano formulations. The improved wound healing performance suggests that NLC-based gel systems offer a promising strategy to enhance therapeutic outcomes and reduce the burden of diabetic wound complications, supporting the development of more effective and accessible healthcare solutions.

Table-2: Wound Area Measurement (% wound closure)

Day	Negative Control (mean ± SD)	Positive Control (Standard) (mean ± SD)	Gel Extract (mean ± SD)	Gel NLC (mean ± SD)
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1	1.26 ± 0.42	0.88 ± 0.02	1.53 ± 0.53	0.90 ± 0.01
2	3.52 ± 1.71	15.17 ± 4.07	13.12 ± 1.54 $^{\beta*}$	11.04 ± 0.41$^{\beta*}$
3	3.02 ± 1.28	26.28 ± 4.13	15.56 ± 1.06 $^{\alpha\beta*}$	24.49 ± 2.33 $^{\beta*}$
4	5.04 ± 1.12	40.33 ± 2.43	25.91 ± 0.45 $^{\alpha\beta*}$	43.89 ± 2.43 $^{\beta*}$
5	5.81 ± 0.88	52.34 ± 1.36	32.30 ± 2.49 $^{\alpha\beta*}$	55.23 ± 2.21 $^{\beta*}$
6	7.58 ± 1.32	71.04 ± 2.49	47.86 ± 1.06 $^{\alpha\beta*}$	68.05 ± 3.70 $^{\beta*}$
7	9.09 ± 1.32	100	66.47 ± 1.67 $^{\alpha\beta*}$	100 $^{\beta*}$
8	10.10 ± 1.12		72.56 ± 0.95 $^{\alpha\beta*}$	
9	12.88 ± 1.98		100 $^{\alpha\beta*}$	
10	16.16 ± 1.10			

Data are presented as mean ± SD (n = 4 wounds). (α, β, *) indicates significant differences ($p<0.05$) compared to the Sanoskin group, negative control, and normal group, respectively.

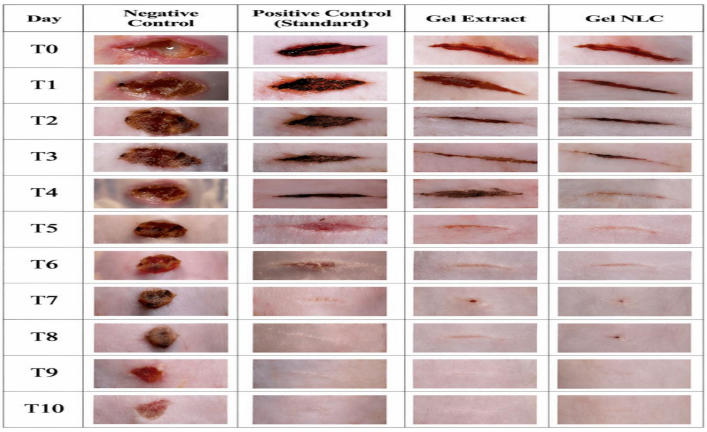

Fig.-3: Macroscopic Observation of Wound Healing Progression Over Time (T0–T10)

CONCLUSION

This study successfully developed and optimised a Nanostructured Lipid Carrier (NLC)-based gel containing combined extracts of *Curcuma longa* and *Syzygium polyanthum*, exhibiting favourable physicochemical properties, nanoscale particle size, and good stability. The NLC gel demonstrated enhanced drug release and permeation compared to the conventional extract gel. In vivo evaluation confirmed superior wound-healing activity, with faster wound contraction and earlier complete closure in diabetic models. These findings indicate that NLC encapsulation improves the delivery and therapeutic efficacy of herbal extracts, making it a promising approach for topical diabetic wound management. Future studies are recommended to explore long-term stability and clinical applicability in human subjects. This study contributes to addressing the global chronic disease burden and aligns with Sustainable Development Goal 3 (Good Health and Well-being) by advancing accessible healthcare solutions for diabetic wound treatment.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-Being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

Tamia Rizqi Novianti http://orchid.org/0009-0004-2184-7910
Herni Kusriani http://orchid.org/0000-0003-4778-6576
Garnadi Jafar http://orchid.org/0000-0001-5659-140X

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