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Formulation and Evaluation of Topical Delivery Diosgenin Emulgel for Diabetic Wounds

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Abstract

A variety of cellular processes work together in a highly coordinated manner to facilitate a complex and dynamic process of wound healing ensuring the efficient restoration of injured tissue. Diosgenin, a plant sterol saponin is primarily found in various plants. The aim of this research was to create an emulgel containing Diosgenin and examine its effects on wound healing in diabetic rats with excision wounds. Histopathological findings further supported the efficacy of the emulgel and results indicate that the application of Diosgenin Emulgel (DE) shows an effective approach for healing of diabetic wounds. The objective of the research stands to explore the possibility of formulation development and wound-healing capabilities of DE**.** The study evaluated the wound healing effects of the DE in Streptozotocin-induced (STZ) (55 mg/kg) given by intraperitoneal route diabetes and control group. The emulgel was topically applied to assess its effectiveness in promoting wound healing. The application of DE on the rat wounds resulted in a notable wound closure within a 21-day period and significant epithelization was observed with $p < 0.001$. The study concluded that the formulation demonstrated remarkable effectiveness in promoting the functional recovery of diabetic wounds.

Keywords: Diosgenin, Emulgel, Excision Wound Model, Streptozotocin (STZ)

1. Introduction

Diabetes Mellitus (DM) is a long-lasting metabolic condition that is becoming more common. Around 25% of individuals with DM experience impaired healing of wounds, particularly in the case of diabetic wound[1]. A complex system is formed through the interaction of different mediators, biomarkers, growth factors, and substages[2]. Haemostasias, swelling, propagation, and dermal renovation are four key phases of wound repair. These phases work together to restore the structure and function of the wound bringing about both physical and physiological recovery[3]. The initial stage of acute wound healing involves the activation of platelets that gather at the injured site and form blood clots and release signaling substances to attract innate immune cells and factors. This process aids in enhancing vascular permeability and initiating the recruitment of necessary components for

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healing[4]. Neutrophils are initial compartments towards enter the looped after bloodstream, followed by pro-inflammatory macrophages that act crucial part trendy to the development of phagocytosis[5]. Long-lasting injuries exhibit a persistent inflammatory phase characterized by diminished levels of growth factors, uncontrolled protease activity, and an elevated presence of bacteria[6].

Development of Granulation tissue, re-growth of epithelial cells, and contraction of wound are all crucial processes of the proliferative phase that are all modified[7]. The stage of maturation where the balance between collagen deposition and breakdown occurs. Fibroblasts bind together the collagen strands in scar tissue to help it contract through their movement[8]. The cost of treatment is influenced by factors such as the extent and duration of the wound progression as well as the rate at which it heals[9]. Throughout history, natural remedies have often offered solutions to various ailments encountered by humankind and wounds are no exception to this pattern[10]. The current research on wound healing showcases the potential of various formulations in managing the healing process and reducing patient distress[11].

Currently, compounds of natural origin have attracted more attention for the development of formulations that can address diabetic wounds. Numerous natural compounds have emerged as significant contributors in this fascinating process[12]. Diosgenin is a steroidal saponin that occurs naturally in various herbal medicinal plants through the hydrolysis of dioscin[13]. Owing to its extensive pharmacologic effects besides therapeutic belongings the situation takes employed for the treatment of cancer, high cholesterol levels, inflammatory conditions, and infections[14]. This compound possesses properties that help reduce inflammation and counteract oxidative stress. It has the potential to provide advantages for various conditions such as diabetes, obesity, abnormalities in blood and brain function as well as allergic diseases[15]. It occurs naturally in different plants, particularly in the saponins of *Dioscorea alata, Smilax china* and *Costus speciosus*[16]. Over time scientists have created diosgenin carbohydrates as well as artificial steroidal saponins primarily for their anti-inflammatory properties[17]. As lipophilic medications face challenges with solubility, it is not feasible to create hydrogels for their synthesis. Consequently, emulgel offers a more reliable and efficient means of releasing lipophilic drugs compared to a basic hydrogel matrix[18]. Thus, the goal of the study was to develop Diosgenin Emulgel (DE) and evaluate its capacity to promote helical growth in diabetic rats that were given Streptozotocin (STZ).

2. Material and Methods

STZ was purchased from Sigma-Aldrich, USA. Diosgenin was obtained from YUCCA Enterprises, Mumbai. The other chemicals and reagents have been procured since Loba Chemie Pvt. Ltd, Mumbai, India.

2.1 Chemicals and Reagents

2.1.1 Animals

Male Wistar rats (180-250 g) were purchased from Global BioResearch Solutions Private Limited, Pune, India, and acclimatized for one week in a housing facility. The study was approved by the animal ethics committee with the number PCP/IAEC/2023/2-6.

2.1.2 Characterization of Diosgenin

Physical Appearance as pH, color, phase separation, solubility, and melting point of the DE were evaluated and analyzed[19].

2.1.3 UV Spectra

A solution of Diosgenin 10 mg in 10 ml methanol was prepared and using a UV-VIS spectrophotometer that scanned the solution in the wavelength range of 200-600 nm to identify the peak absorption point[20].

2.1.4 Formulation of Emulgel

Emulgel was prepared using a modified version of the process mentioned by Ali Khan B[21]. The gel part of the emulgel was created by dissolving carbopol-934 in cold water and stirring constantly at a moderate speed until a homogenous mixture was formed. The pH was then determined. Triethanolamine (TEA) was used to adjust the pH to 6-6.5. Tween 80 was dissolved in distilled water to prepare the aqueous phase of the emulsion, while span 20 was dissolved in liquid paraffin to prepare the oil portion of the emulsion. To maintain the emulsion, methylparaben was dissolved in propylene glycol and the DE in ethanol before being combined with the aqueous phase. Separately, the aqueous and oil phases were heated in a water bath at 70°C. The oil phase was then added drop by drop to the aqueous phase while continuously stirring for 10 minutes before being cooled to room temperature. Finally, the gel and emulsion components were mixed in a 1:1 ratio with moderate stirring to make emulgel as depicted in Table 1.

Table 1. Composition of various emulgel formulations

Ingredients (%w/w)	F1	F2
diosgenin	0.5	1
Carbapol - 934	0.5	1
Tween 80	0.5	0.5
Span 20	1	1
Liquid paraffin	7.5	7.5
Propylene glycol	5	5
Methyl paraben	0.03	0.03
Distilled water q.s	100	100

2.2 Stability Study

The stability of the DE formulation was evaluated and tested across different temperatures in addition to moistness specifically at 8°C, 25°C, 40°C and 40°C ± 75 % Relative Humidity (RH). An organoleptic evaluation of the emulgel was conducted to observe any potential alterations in its color, odor, consistency and microbial culture characteristics. The pH levels of freshly prepared emulgel and storing circumstances (8°C, 25°C, and 40°C) were measured.

2.3 *In-Vitro* Anti-Microbial Activity

Escherichia coli and *Staphylococcus aureus* the commonly occurring bacteria causing skin infections were obtained from the Department of Microbiology, Poona College of Pharmacy, Pune selected for the study[22]. *In-vitro* antibacterial activity was evaluated using the Muller-Hinton agar well diffusion technique[23].

2.4 Induction of Diabetes

Diabetes was induced by injecting Nicotinamide (NA) (200 mg/kg) via intraperitoneal injection immediately followed by STZ (55 mg/kg) within a span of 15 minutes[21]. After a period of 72 hours, the blood glucose levels were measured using the OneTouch Plus® Blood sugar Demonstrator. Animals with blood glucose levels exceeding 250 mg/dL were selected as the subjects for the wound model[24].

2.5 Experimental Design

Animal grouping took place 72 hours after the administration of STZ. Animals were alienated into 5 clusters (n = 6) in a random manner. Normal Control group (NC), Diabetes Control group (DC), diabetic animals treated with Povidone Iodine (5%) diabetic animals treated with low dose (LD-0.5%) DE, Diabetic animals treated with high dose (HD-1.0%) DE respectively as group I, II, III, IV, V.

2.6 Excision Wound Model

The rats underwent anesthesia through an intraperitoneal injection of ketamine (80 mg/kg). The back of the rats was cleaned and shaved[24]. In a sterile environment incision of 10 mm was created in the skin and the excised piece was removed. The areas of the wounds were measured and documented on days 0, 7, 14 and 21 for all experimental groups.

2.7 Histopathological Investigation

The wound samples were preserved in 10% buffered formalin and then embedded in paraffin following the normal test site procedures aimed at histopathology examination. Tissue sections of 5 µm width remained hydrated, marked with hematoxylin and eosin (H and E).

2.8 Statistical Analysis

The findings were reported as the standard deviation using Graph Pad Prism 7 ®. A two-way ANOVA was employed to assess the significance of variances. Throughout all the experiments statistical significance was defined as $p < 0.05$.

3. Results

3.1 Preformulation Study of Diosgenin

A preformulation assessment of the diosgenin was conducted for color, solubility, and melting point. It appears as a white substance and no phase separation was detected. It exhibits low solubility in water but shows partial solubility in methanol and ethanol. However, the situation was completely resolved in benzene and chloroform. The melting point of diosgenin is recorded at 205.5 °C.

3.2 UV Spectra

The Diosgenin solution in methanol underwent a UV scan, revealing a maximum value of 432.7 nm. In the process of preparing the calibration curve for diosgenin, methanol was used as the solvent. The concentration of diosgenin was systematically increased and a regression equation was derived from the data. The correlation coefficient obtained for the calibration curve in methanol was found to be 0.9971 as per Figure 1.

3.3 Formulation and Evaluation of DE

Emulgel formulations F1 and F2 were developed using Carbopol 940 polymer and subjected to evaluation based on physical appearance, pH, and anti-microbial activity. Following the assessment of these parameters, the emulgel was chosen for further evaluation that included measuring viscosity, drug content, and *in vitro* diffusion profile. The pH values of both F1 and F2 were within the neutral pH range (6.8-7.5) indicating that they are unlikely to cause skin irritation. Formulation F1 exhibited a white, creamy appearance while F2 appeared as an off-white, homogenous product without any gritty texture.

3.4 Stability of DE

Stability was checked for a duration of 3 months while being stored at various temperatures including 8°C, 25°C, 40°C, 40°C + 75 % RH. The pH values of formulations were regularly monitored at different time intervals throughout the period. Physical incompatibility was not found suggesting the diosgenin stability in the emulgel. Further, there were no changes in pH, color, odor, and physical appearance. This depicts that emulgel maintained its homogeneity without any alterations[25].

3.5 Viscosity, Spreadability, Extrudability and Drug Content Parameters of DE

Based on the pH, physical appearance, and stability emulgels were evaluated in terms of anti-microbial activity and *in vivo* study. The Carbopol 940 concentration in the formulation was 1g, the viscosity remained consistent without any variation, and the observed value 0.38 poise which is considered ideal for a topical gel formulation. The spreadability value of 32.46 indicated that the emulgel could be easily spread on the skin. The emulgel exhibited excellent extrudability. The drug content in the gel was determined to be 95.23% w/w.

3.6 Antimicrobial Activity

The antimicrobial activity of DE against microorganisms was examined and their potency was assessed by the presence or the absence of inhibition zones. The results showed that 1% concentration DE the inhibition zone was the maximum as compared to 0.5% concentration. The result of the present study suggests that the DE is more active in 1% concentration gel. For F1 formulation the observed zones of inhibition against *E. coli* and *S. aureus* were 2.0 and 1.4 mm respectively. Formulation F2 showed inhibition of 1.4 and 1.0 mm against the same bacteria.

3.7 Conclusion of DE on the Histological Analysis of Injured Tissue in Experimental Rats

Histological analysis 0, 7th, 14^{th,} and 21st day observed in Figure 2. In Group II the histopathological examination revealed the absence of an abundant cuticular slip layer compared to Group I. However, in Group III, IV and V there was a noticeable improvement in re-epithelialization. These treated groups also showed significant repair of fine-developed mature granulation

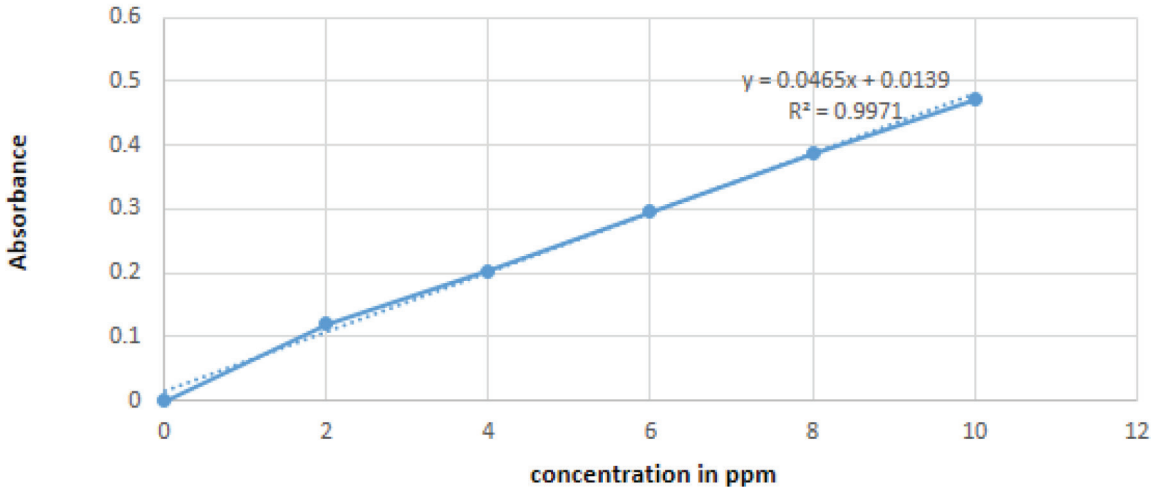


Figure 1. Standard diosgenin curve at 433 nm.

tissue with an abundance of original vessels and fibrosis inside the dermis along again dumped collagen fibers. Histological analysis indicated that Group III, IV, and V rats exhibited an overall earlier recovery and regeneration compared to Group II.

3.8 Effect of DE on Wound Size Reduction in mm and % in Experimental Rats

The results presented are from normal control and diabetic experimental rats. The constraint deliberates on minimizing of injured size in mm since preliminary injury. The situation was detected that the percentage of injury reduction in Group II was reduced compared with Group I. This result remained noticeable from 7th day ahead. When compared to Group II, the experimental rats in Groups IV and V that received topical LD and HD demonstrated considerable wound healing by the VII day as shown in Table 2 and Figure 3.

4. Discussion

The primary objective of this research was to assess the efficacy of diosgenin-based emulgel in accelerating wound healing. Emulgel possesses the benefits of both gels and emulsions, serving as a drug reservoir, where emulsion droplets enable the encapsulation of hydrophobic drugs and enhance their penetration through the skin. Preformulation evaluations were executed on diosgenin, encompassing the assessment of its color, solubility, melting point, and UV properties. A significant focus was placed on compounds that showed robust absorption across the entire ultraviolet spectrum during the analysis of UV properties. This concentration assessment was carried out using a standard curve that allowed for precise measurement of diosgenin levels. Stability studies conducted on diosgenin emulgel formulations demonstrated that the drug retained its integrity.

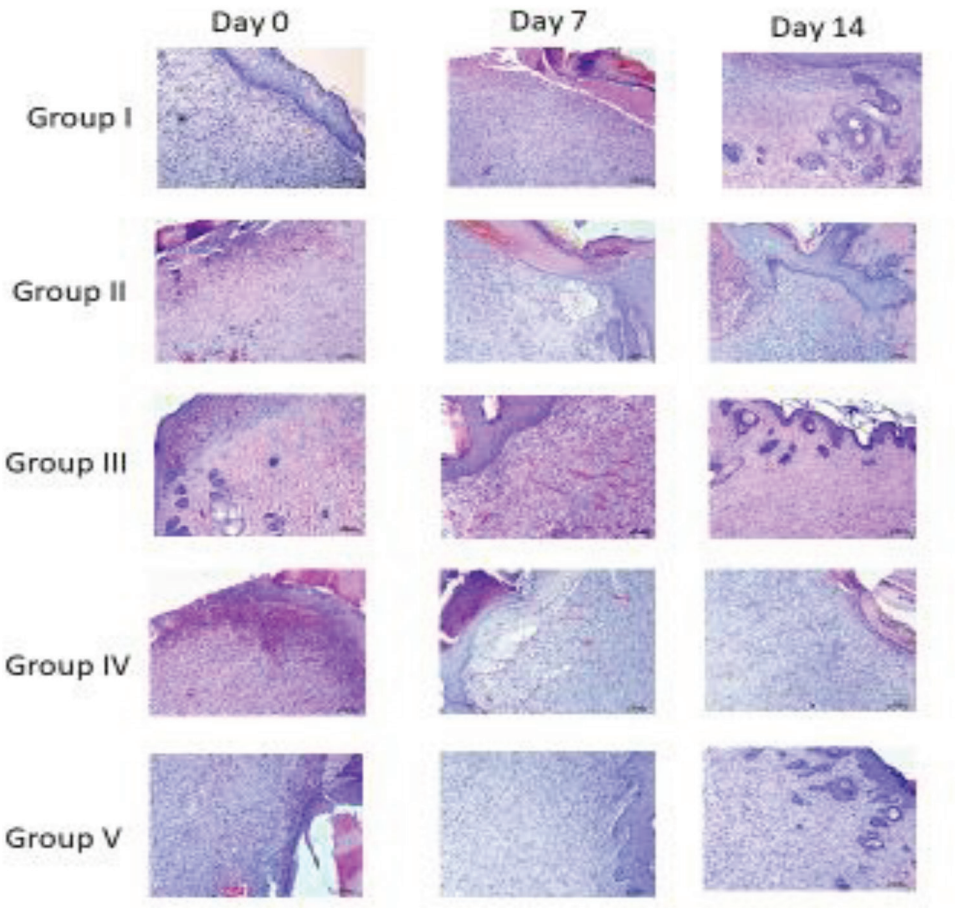


Figure 2. Histopathological changes on skin wound in STZ- induced diabetic rats stained with Haematoxylin-Eosin. Original magnification 400x (the scale bar is 100 μm).

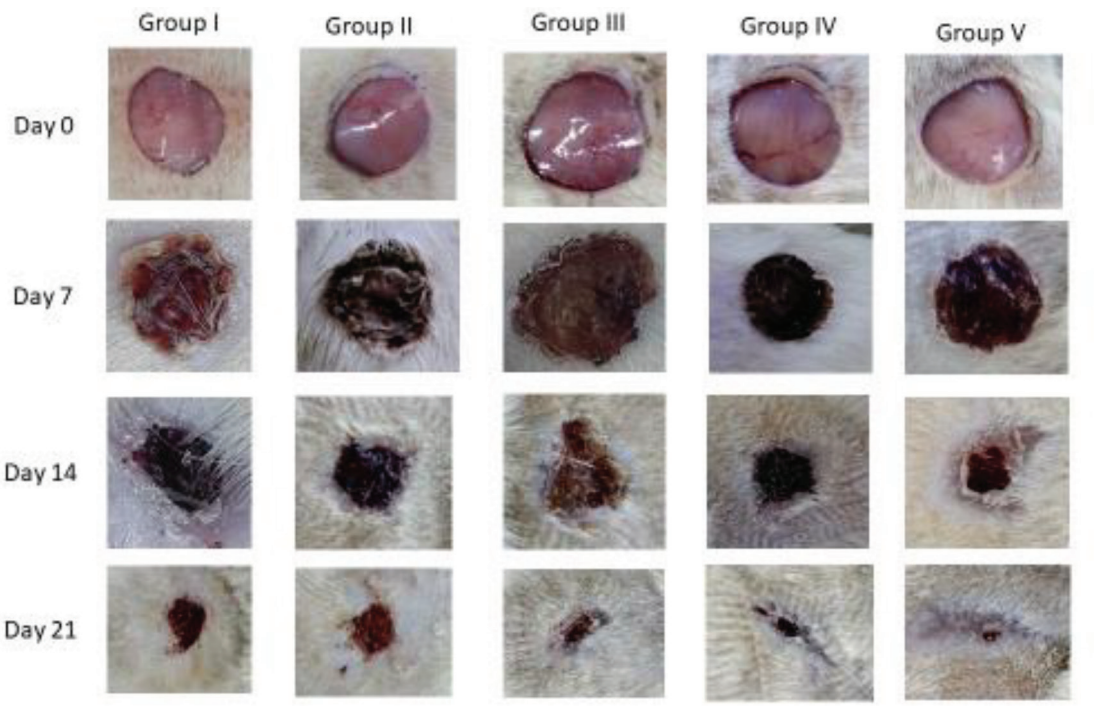


Figure 3. The topical application of DE and its impact on the healing of an excision wound model on various days (0, 7, 14, and 21) (Digital camera used (Sony, 7.2 megapixel), Japan.

Table 2. Excision wound study showing a reduction in wound size (mm)

	Day 0	Day 7	Day 14	Day 21
Normal Control (NC)	10 ± 0.12	8.62 ± 0.13	6.44 ± 0.13	4.45 ± 0.08
Disease Control (DC)	10 ± 0.11	8.74 ± 0.07	7.37 ± 0.10	6.29 ± 0.06
Standard (STD)	10 ± 0.20	7.82 ± 0.04	5.70 ± 0.05	3.17 ± 0.04
Low Dose (LD)	10 ± 0.10	8.00 ± 0.04	5.79 ± 0.10	3.28 ± 0.13
High Dose (HD)	10 ± 0.10	7.61± 0.06	5.18 ± 0.08	2.79 ± 0.17

The evaluation of the emulgel's various characteristics, including color, grittiness, phase separation, spreadability, pH, and viscosity, is essential for assessing its quality. Additionally, an appropriate agitation speed must be used during the process to ensure the stability of the formulation components and prevent coalescence. This is a critical factor that can affect the overall integrity of the emulgel.

It is essential to identify a suitable dosage form that can effectively distribute diosgenin topically while ensuring its stability and controlled release. The results of this study revealed that the diosgenin emulgel formulation displayed promising characteristics in terms of formulation and skin compatibility, suggesting that it could enhance the patient acceptance of treatment.

The emulgel demonstrated favorable spreadability and exhibited excellent extrudability. The viscosity of the emulgel had an inverse relationship with its spreadability that is higher viscosity led to increased retention of the emulgel on the skin. Formulation F2 showed relatively lower activity against *S. aureus* and *E. coli* while formulation F1 demonstrated greater activity.

The utilization of animal models is prevalent in wound research, as they are employed to simulate human wound healing issues, such as burns, dehiscence, ischemia, venous or pressure ulceration, infection, and scarring[26]. Additionally, animal models have been used to study the molecular mechanisms of acute wound healing, which cannot be determined through human research[27]. Wound healing is a complex process involving cell and Extracellular Matrix (ECM) interactions that can affect various hard and soft tissues and organ systems. The normal mammalian response to cutaneous injury includes inflammation, granulation

tissue formation, epithelialization, and remodeling of new tissue to restore tissue integrity[28]. The use of an *in vivo* model is necessary when attempting to obtain information on the multifactorial nature of the wound healing process, which may be influenced by externally introduced factors or the presence of an underlying pathology[29].

The use of rats in the investigation of skin wound healing and the assessment of various treatment options, such as the diosgenin emulgel formulation, has been common. Rats are often chosen because of their accessibility, affordability, and small size. Incisional and excisional models are commonly used, in which the wound is situated on the dorsum of a rat. This location is advantageous in preventing the animal from accessing and manipulating the wound. Rats and humans share similar skin characteristics, including the presence of epidermis, basement membrane, hair follicles, and dermis[30].

In the present investigation, we opted to utilize the excision wound model due to its proven reliability. Research has demonstrated that excisional wounds exhibit a more consistent healing pattern than incisional wounds, rendering them an ideal choice for our study. Furthermore, excisional wounds display healing profiles that closely resemble those observed in human wound healing, which is particularly relevant considering our use of diabetic animals in this research[31]. Diabetes, as a known impediment to the wound-healing process, necessitated the use of this model[32].

In the study, it was observed that the diabetic rats treated with DE exhibited faster healing of excision wounds compared to the control group. Additionally, histopathological analysis of skin wounds in the DE (Group IV, V) and Providon Iodine (STD Group III) treated groups on day 14 revealed healed skin structures characterized by normal epithelization, restored adnexa, and fibrosis within the dermis as depicted in Figure 4. However, the diabetic control group (Group II) showed delayed formation of ground substance in the granulation tissue. The mechanism by which DE promotes more rapid wound contraction could be attributed to its ability to stimulate the production of beneficial interleukins and IFN-γ production while simultaneously inhibiting harmful interleukins[33,34]. DE has been shown to affect the function and recruitment of various inflammatory cells, fibroblasts, and keratinocytes through its influence on an inflammatory α-chemokine. In addition, DE may increase gap junctional intracellular communication in fibroblasts and induce a more rapid maturation of granulation tissue, leading to faster wound contraction. This may be due to the anti-inflammatory action of DE. Diosgenin has been found to exhibit immune-protective and antioxidant properties, which help prevent lipid peroxidation. Additionally, it reduces inflammation and promotes wound healing.

The histological analysis indicated a greater degree of cellular infiltration in the treated instances, as

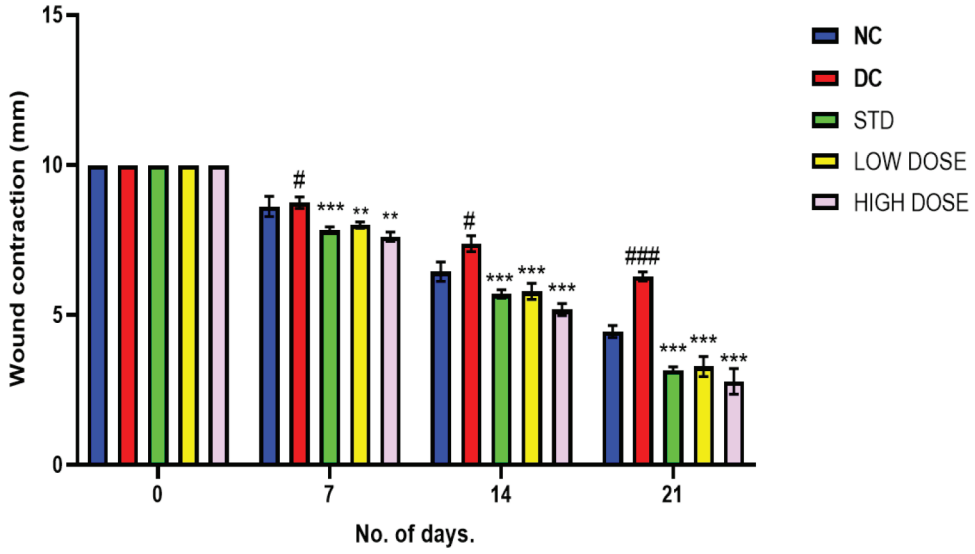


Figure 4. Effect of DE on wound size (mm) in STZ- induced diabetic rats (as mean ± SEM (n = 4) and analyzed by two-way ANOVA followed by Bonferroni multiple comparison test. $^{\#}p<0.05$, $^{\#\#}p<0.01$ as compared to NC groups. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ as compared with DC group.

observed through hematoxylin and eosin staining. The administration of diosgenin emulgel demonstrated beneficial effects on early dermal and epidermal regeneration in rats by promoting cellular proliferation, granular tissue development, and epithelization. The emulgel formulations containing active diosgenin contributed to wound healing by facilitating the regeneration and organization of new tissues. From the conclusion of the experiment, it was evident that treatment with diosgenin emulgel was nearly complete. Studies have provided evidence supporting the efficacy of wound healing medication in repairing endothelial function and accelerating tissue regeneration for the treatment of skin lesions. This research has confirmed that diosgenin exhibits promising wound-healing properties and highlights the potential of diosgenin emulgel as a valuable tool for evaluating wound-healing processes.

The findings of this research paper provide valuable insights into the potential benefits of Diosgenin emulgel formulation in promoting diabetic wound healing. The experimental results demonstrated that the application of the emulgel formulation on excision wounds in diabetic animal models significantly accelerated the wound closure process compared to the disease group. The formulation exhibited remarkable effects on improving various wound healing parameters including reduced wound area, enhanced epithelialization, increased collagen deposition and decreased inflammatory response. These positive outcomes suggest that diosgenin emulgel holds great promise as a therapeutic approach for diabetic wound management offering new possibilities for improved patient outcomes and quality of life.

5. Study Limitations

The investigation was conducted on animal models and primarily focused on excision wounds that may not fully represent the complexity and diversity of real-life wound scenarios. Moreover, the research did not explore the long-term effects of the DE formulation, necessitating human extrapolation.

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7. References

1. Burgess JL, Wyant WA, Abujamra BA, Kirsner RS, Jozic I. Diabetic wound-healing science. Medicina. 2021; 57:1072-95. https://doi.org/10.3390/medicina57101072
2. Redondo MJ, Hagopian WA, Oram R, Steck AK, Vehik K, Weedon M. The clinical consequences of heterogeneity within and between different diabetes types. Diabetologia, Springer. 2020; 63:2040-8. https://doi.org/10.1007/s00125-020-05211-7
3. Dong J, Chen L, Jayaswal N, Meghani I, Zhang W, Veves A. Mast cells in diabetes and diabetic wound healing. Israel Deaconess Foot Center JB. 2020; 37:4519-37. https://doi.org/10.1007/s12325-020-01499-4
4. Deng L, Du C, Song P, Chen T, Rui S, Armstrong DG, Deng W. The role of oxidative stress and antioxidants in diabetic wound healing. Oxidative Medicine and Cellular Longevity. Hindawi Limited. 2021; 1-11. https://doi.org/10.1155/2021/8852759
5. Bodas K, Shinde V. Healing of wounds: A detailed review on models, biomarkers, biochemical and other wound assessment parameters. International Journal of All Research Education and Scientific Methods (IJARESM). 2021; 9(3):2069-85.
6. Wilkinson HN, Hardman MJ. Wound healing: Cellular mechanisms and pathological outcomes. Open Biology. Royal Society Publishing. 2020; 10:200-23. https://doi.org/10.1098/rsob.200223
7. Raziyeva K, Kim Y, Zharkinbekov Z, Kassymbek K, Jimi S, Saparov A. Immunology of acute and chronic wound healing. Biomolecules. 2021; 11:700-24. https://doi.org/10.3390/biom11050700
8. Al Sadoun H. Macrophage phenotypes in normal and diabetic wound healing and therapeutic interventions. Cells. 2022; 11:2430-57. https://doi.org/10.3390/cells11152430
9. Alam W, Hasson J, Reed M. Clinical approach to chronic wound management in older adults. Journal of the American Geriatrics Society. 2021; 69:2327-34. https://doi.org/10.1111/jgs.17177
10. Liang Y, Li M, Yang Y, Qiao L, Xu H, Guo B. pH/Glucose dual responsive metformin release hydrogel dressings with adhesion and self-healing via dual-dynamic bonding for athletic diabetic foot wound healing. ACS Nano. 2022; 16(2):3194-207. https://doi.org/10.1021/acsnano.1c11040
11. Prasetyono TOH. General concept of wound healing, revisited. Med J Indones. 2009; 18:208-16. https://doi.org/10.13181/mji.v18i3.364
12. Zhou X, Ruan Q, Ye Z, Chu Z, Xi M, Li M, Hu W, Guo X, Yao P. Resveratrol accelerates wound healing by attenuating oxidative stress-induced impairment of cell proliferation and migration. Burns. 2021; 47(1):133-9. https://doi.org/10.1016/j.burns.2020.10.016

13. Arya P, Munshi M, Kumar P. Diosgenin: Chemistry, extraction, quantification and health benefits. Food Chemistry Advances. 2023; 2:100170. https://doi.org/10.1016/j.focha.2022.100170
14. Varghese R, Shinde V. Therapeutic potential of novel phyto-medicine from natural origin for accelerated wound healing. International Journal of Pharmacognosy. 2021; 8(1):14-24.
15. Tariq M, Tahir HM, Butt SA, Ali S, Ahmad AB, Raza C, Summer M, Hassan A, Nadeem J. Silk-derived formulations for accelerated wound healing in diabetic mice. Peer J. 2021; 9:1-18. https://doi.org/10.7717/peerj.10232
16. Bodas KS, Bagul CD, Shinde VM. Evaluation of wound healing effect *of Mallotus philippensis* (Lam.) Mull Arg by *in silico* multitargets directed for multiligand approach. *In Silico* Pharmacol. 2022; 10(1):1-19. https://doi.org/10.1007/s40203-022-00134-0
17. Parama D, Boruah M, Yachna K, Rana V, Banik K, Harsha C, Thakur K, Dutta U, Arya A, Mao X, Ahn K, Kunnumakkara A. Diosgenin, a steroidal saponin, and its analogs: Effective therapies against different chronic diseases. Life Sciences. 2020; 260-87. https://doi.org/10.1016/j.lfs.2020.118182
18. Okonogi S, Riangjanapatee P. Physicochemical characterization of lycopene-loaded nanostructured lipid carrier formulations for topical administration. Int J Pharm. 2015; 478(2):726-35. https://doi.org/10.1016/j.ijpharm.2014.12.002
19. Ambala R, Vemula SK. Formulation and characterization of ketoprofen emulgels. J Appl Pharm Sci. 2015; 5(7):112-7. https://doi.org/10.7324/JAPS.2015.50717
20. Nazir R, Pandey DK, Pandey B, Kumar V, Dwivedi P, Khampariya A, Dey A, Malik T. Optimization of diosgenin extraction from *Dioscorea deltoidea* tubers using response surface methodology and artificial neural network modeling. PLoS One. 2021; 16:1-19. https://doi.org/10.1371/journal.pone.0253617
21. Khan BA, Ullah S, Khan MK, Alshahrani SM, Braga VA. Formulation and evaluation of *Ocimum basilicum*-based emulgel for wound healing using an animal model. Saudi Pharm J. 2020; 28(12):1842-50. https://doi.org/10.1016/j.jsps.2020.11.011
22. Charyulu NR, Joshi P, Dubey A, Shetty A. Emulgel: A boon for enhanced topical drug delivery. Journal of Young Pharmacists. 2021; 13(1):76-9. https://doi.org/10.5530/jyp.2021.13.17
23. Khan BA, Ali A, Hosny KM, Halwani AA, Almehmady AM, Iqbal M, Alharbi W, Abualsunun A, Bakhaidar R, Murshid S, Khan M. Carbopol emulgel loaded with ebastine for urticaria: Development, characterization, *in vitro* and *in vivo* evaluation. Drug Deliv. 2022; 29(1):52-61. https://doi.org/10.1080/10717544.2021.2015483
24. Rahman M, Ahmad Bhat K, Ara T. Synthesis and antimicrobial activity of triazolyl analogs of diosgenin. The Journal of Phytopharmacology. 2017; 6(4):227-33. https://doi.org/10.31254/phyto.2017.6405
25. Choudhry A, Akhtar N. Formulation, characterization of *Quercus infectoria* (Olivier) emulsions, and *in vitro, in vivo* evaluation as cosmeceutical formulation. J Cosmet Dermatol. 2023; 22(12):3480-90. https://doi.org/10.1111/jocd.15865
26. Gottrup F, Ågren MS, Karlsmark T. Models for use in wound healing research: A survey focusing on *in vitro* and *in vivo* adult soft tissue. Wound Repair and Regeneration. 2000; 8(2):83-96. https://doi.org/10.1046/j.1524-475x.2000.00083.x
27. Masson-Meyers DS, Andrade TAM, Caetano GF, Guimaraes FR, Leite MN, Leite SN, Frade MAC. Experimental models and methods for cutaneous wound healing assessment. Int J Exp Pathol. 2020; 101(1-2):21-37. https://doi.org/10.1111/iep.12346
28. Cross SE, Naylor L, Coleman RA, Teo TC. An experimental model to investigate the dynamics of wound contraction. British journal of plastic surgery. 1995; 48(4):189-97. https://doi.org/10.1016/0007-1226(95)90001-2
29. Franz MG, Smith PD, Wachtel TL, Wright TE, Kuhn MA, Ko F, Robson MC. Fascial incisions heal faster than skin: A new model of abdominal wall repair. Surgery. 2001; 129(2):203-8. https://doi.org/10.1067/msy.2001.110220
30. Dorsett-Martin WA. Rat models of skin wound healing: A review. Wound Repair Regen. 2004; 12(6):591-9. https://doi.org/10.1111/j.1067-1927.2004.12601.x
31. Jung DH, Park HJ, Byun HE, Park YM, Kim TW, Kim BO, Um SH, Pyo S. Diosgenin inhibits macrophage-derived inflammatory mediators through downregulation of CK2, JNK, NF-κB, and AP-1 activation. International Immunopharmacology. 2010; 10(9):1047-54. https://doi.org/10.1016/j.intimp.2010.06.004
32. Leite SN, Leite MN, Caetano GF, Ovidio PP, Junior AA, Frade MA. Phototherapy improves wound healing in rats subjected to a high-fat diet. Lasers in Medical Science. 2015; 30:1481-8. https://doi.org/10.1007/s10103-015-1745-2
33. Fisman EZ, Motro M, Tenenbaum A. Cardiovascular diabetology in the core of a novel interleukins classification: The bad, the good and the aloof. Cardiovascular Diabetology. 2003; 2(1):1-0. https://doi.org/10.1186/1475-2840-1-2
34. Ku CM, Lin JY. The anti-inflammatory effects of 27 selected terpenoid compounds were tested through modulating Th1/Th2 cytokine secretion profiles using murine primary splenocytes. Food Chemistry. 2013; 141(2):1104-13. https://doi.org/10.1016/j.foodchem.2013.04.044