RJNMSR
Review Journal of Neurological & Medical Sciences Review

Volume 3, Issue 3, 2025

SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL EVALUATION OF CURCUMIN-LOADED NANOETHOSOMES

[1]Waqas Hussain, [1*]Muhammad Tariq, [2]Aasma Akram, [3]Danyal Iftikhar, [4]Nayla Javed, [5]Sadia Rehman, [6]Mubashir Ali Khalique, [7,8]Usman Javaid, [9,10]Faiza Mumtaz, [11,12*]Hafiz Muhammad Usman Abid

[1]*Faculty of Pharmaceutical Sciences, Times University, Multan, Pakistan*
[2]*Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Government College University, Faisalabad 38000, Pakistan*
[3]*Department of Pharmacology, School of Basic Medical Sciences, Xi'an Jiaotong University, Xi'an, 710061, China.*
[4]*Department of Pharmaceutics, Faculty of Pharmacy, The Islamia University of Bahawalpur, Pakistan*
[5]*Riphah Institute of Pharmaceutical Sciences, Riphah International University Sahiwal campus, Pakistan*
[6]*Faculty of Veterinary and Animal Sciences, University of Poonch, Rawalakot, Pakistan*
[7]*Faculty of Pharmacy and Pharmaceutical Sciences, Department of Pharmacology, University of Karachi,*
[8]*KAIMS International Institute, Multan, Pakistan*
[9]*Department of Pharmacology, Tehran University of Medical Sciences, Tehran, Iran,*
[10]*Lyallpur College of Pharmaceutical Sciences, Faisalabad, Pakistan*
[11*]*Department of Pharmaceutics, Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan,*
[12*]*Health Services Academy, Islamabad, Pakistan.*

Corresponding Authors: *
Muhammad Tariq & Hafiz Muhammad Usman Abid
drmuhammadtariq@t.edu.pk, usman.abid@hsa.edu.pk

DOI: https://doi.org/10.5281/zenodo.16422637

Received	Accepted	Published
14 June, 2025	19 July, 2025	25 July, 2025

ABSTRACT

Diabetic wounds represent a serious and growing global health challenge due to their slow healing, high risk of infection, and potential for severe outcomes such as limb amputation. The increasing prevalence of diabetes worldwide underscores the urgent need for effective treatment strategies for chronic wounds. Curcumin, a bioactive compound derived from Curcuma longa, has shown considerable therapeutic potential in wound healing owing to its anti-inflammatory, antioxidant, and antimicrobial properties. However, its clinical application is limited by poor aqueous solubility, low bioavailability, and restricted permeability across biological membranes. To address these limitations, the present study investigated the development and therapeutic efficacy of curcumin-loaded nanoethosomes, a type of lipid-based nanocarrier designed to improve the delivery of poorly soluble compounds. The antioxidant capacity of the nanoformulations were assessed using the DPPH free radical scavenging assay, and results indicated that curcumin-loaded nanoethosomes exhibited significantly higher antioxidant activity compared to free curcumin, suggesting improved stability and radical neutralization ability. The in vivo efficacy of the nanoethosomal formulation was evaluated in a diabetic wound rat model. Animals were divided into three groups: control, standard care, and curcumin-loaded nanoethosomes. Over an 11-day period, wound healing parameters such as wound contraction, granulation tissue formation, and epithelialization were monitored. The group treated

with nanoethosomal curcumin showed significantly enhanced healing, with more rapid wound contraction, better granulation tissue development, and faster epithelialization compared to the control and standard care groups. The superior healing effect was attributed to the improved solubility, bioavailability, and sustained release profile of curcumin from the nanoethosomes, which facilitated effective drug delivery to the wound site and reduced oxidative stress. In conclusion, curcumin-loaded nanoethosomes significantly improved diabetic wound healing and represent a promising strategy for future clinical applications. Further studies are needed to optimize the formulation and assess its long-term safety and efficacy in human subjects.

Keywords: *Diabetes, Nanoethosomes, Curcumin, Wound Healing, Bioavailability, Antioxidants.*

INTRODUCTION

Diabetes as a global public health care problem due to increasing incidence rates and complications especially in diabetic complications–chronic wounds. Among the most common and severe comorbidities, there are the diabetic foot ulcers. Such wounds often lead to infections, amputations and a significantly lowered quality of life of the affected individual. Diabetes patients delayed wound healing is attributed to; reduced blood circulation, nerve damage, and increased risk of infection. These elements put together, make it very difficult for wounds to heal on their own, naturally hence the need to come up with modern, powerful and effective therapeutic interventions (Burgess et al., 2021).It is obtained from the root of the plant called Curcuma longa, commonly known as turmeric and is mainly used to treat inflammation, free radical injury and for the healing of wounds. That means curcumin although possesses enormous pharmacological properties and can act as therapeutic agent, it has limited clinical use due to its poor solubility in water, short biological half life and low levels of absorption. Because of these pharmacokinetic challenges, researchers are focusing on development of new approaches to increase the solubility, stability and bioavailability of curcumin thus enhancing its therapeutic efficacy.Nanoethosomes, a new drug delivery system of hydrophobic drugs that are expected to enshrined curcumin in the very near future, has been made feasible through the advancement in the nanotechnology. Nanoethosomes, ethanolic phospholipid vesicles, have proved to be highly effective in enhancing the skin permeability and for the effective deposition of the medicaments at the deeper layers of the skin. There are, however, some limitations associated with curcumin formulations and it is with these in mind that researchers aim at putting the medication in nanoethosomes to ensure that the medication release is controlled and that it remains at the wound site for an adequate amount of time (Nair et al., 2022).This work aims at synthesizing curcumin loaded nanoethosomes for the treatment of diabetic wounds, characterizing these formulations, and evaluating their pharmacological potential. This work aims at trying to explain the enhanced therapeutic potential of curcumin nanoethosomes as opposed to conventional curcumin formulations by employing different in vitro and in vivo models. The ultimate aim is to develop a new therapeutic approach which may help to improve the diabetic wound treatment, reduce rate of complications there and thus, contribute to the improvement of patient's outcome. In the current study, on curcumin nanoethosomal formulation was used which enhances drug solubility and bioavailability and has been found to yield positive results. To the best of my knowledge, there are limited and scarce research that has tested nanoethosome-encapsulated curcumin with normal formulations in diabetic wound healing models. These innovative formulations can be used as potential therapeutic candidates in the management of diabetic wound healing and new developmental studies demonstrate the requirement of detailed pharmacokinetics, therapeutic effect and toxicity studies of these new formulations. To confirm the hypothesis that nanoethosomes could deliver hydrophobic anti-cancer drugs such as curcumin effectively, this gap has to be filled (Karthikeyan et al., 2020).

METHODOLOGY

PREPARATION OF NANOETHOSOMES BY ETHANOL INJECTION METHOD

Nanoethosomes were prepared by the ethanol injection method and the organic phase consisted of 0. 5 w/v cholesterol and 2% w/v phospholipid such as phosphatidyl choline dissolved in 10 mL of ethanol. A suitable buffer solution or distilled water was inferred to prepare the aqueous phase separately and the aqueous phase and it was heated in between 40 and 60°C. Subsequently, 90 ml of the warmed aqueous phase were then dropped slowly with the ethanol-lipid mixture under stirring at a speed of 5000 rpm. The phospholipids form nanostructured ethosomes when the ethanol diffuses in the water. This was done with a view of ensuring that full vesicle formation was achieved through swirling of the mixture for thirty to sixty minutes. To achieve a better particle size distribution, they were homogenised under high pressure and or for 5 to 10 minutes by ultrasonication. The last suspension which was prepared for the experiment was aliquoted and

kept for further use and testing at 4°C (Elsayed et al., 2021).

IN-VITRO ACTIVITIES

FTIR

To identify which functional groups were entrapped in the nanoethosomes formulations a Fourier transform infrared spectroscopy was conducted. The spectra were recorded using a Fourier Transform Infrared (FTIR) spectrometer, at room temperature in the range of 4000- 400 cm^{-1}. Higher signal-to-noise ratio of the obtained spectra was achieved by accumulating 32 scans with a spectral resolution of 4 cm^{-1}. Before each scan a background correction was applied in order to exclude any possible atmospheric interferences like carbon dioxide and water vapor. Thus, when comparing the studied individual component spectra with the nanoethosomes formulation, the specific characteristic absorption bands of various functional groups were identified. This comparison went a long way in confirming this interaction which was between the components. The incorporation of curcumin into the nanoethosomes and the stability of the phospholipid bilayer structure was a crucial factor to confirm and the FTIR data were essential to achieve this.

ANTIOXIDANT ACTIVITY

The antioxidant activity of the in vitro activities was estimated using a free radical scavenging technique. The antioxidant activity was determined with curcumin, nanoethosomes and nano ethosomes of curcumin. The experiment was performed on the samples using the 96 well microplate with a free radical scavenging at a slight modification. After the solutions have been mixed with DPPH ethanol solution, the solutions were diluted. For a control, ethanol blank was used. Each of the sample was tested in triplicate and the absorbance at 517 nm was taken after incubating the samples at room temperature without exposure to light for 90 minutes. The one employed for the evaluation of the radical scavenging activity was I% = [((Abs0 - Abs1)/Abs0)] * 100, whereby Abs0 refers to the blank sample, and Abs1 refers to the sample (Baschieri & Amorati, 2021).

IN-VIVO ACTIVITIES

DIABETIC WOUND HEALING

Thus, in the in vivo study of the efficacy of nanoethosomes containing curcumin to treat wounds in rats induced with dexamethasone diabetes was tested. Wistar rats were used in this study since it was discovered that male albino Wistar rats weighing between 150-200g could be induced into became diabetic after administering dexamethasone (10mg/kg SC for 10 days) injection. This is a solution made from isotonic sodium chloride; it is free from any shading or color The solution has a concentration of 2. This corresponds to 5ml/kg SC for 10 days was Injected intravenously through the tail vein. The rats were housed for some time before diabetes was induced then they were allowed to adjust before the experiment was conducted. For the study, rats with blood glucose level above 250mg/dl after 21 days were used for the study. Unfortunately, four groups of rats only were involved in the wound healing study. To make dorsal wounds each group was provided with the following. Before the procedure the rats were put under anesthesia to ensure that they are comfortable when the procedure was being conducted and to maintain sterility the dorsal area was sanitized and depilated. By attaching a specialised perforator with suction, a circular incision of 2 cm2 was made on the dorsum part of each rat. Once the wound was made, nanoethosomes were administrated on the wound site on the 2nd, 5th, 8th and 11th day. Other parameters measured during the study include wet and dry granulation tissue weights, period of epithelization and percent wound contraction where a Vernier caliper was used in determination of wound contraction (Alsarayreh et al., 2022).

WET AND DRY GRANULATION WEIGHT

On the eleventh day the new granulation tissue formed on the wound site was excised and the wet weight obtained. The tissue carrying the wet granulation was then left in the oven to dry for 24 hours at 60°C After which the weight was again taken.

PERIOD OF EPITHELIALIZATION

It is identified by quantifying the number of days that the eschar takes in order to desquamate leaving no sign of the initial raw wound.

WOUND CONTRACTION IN PERCENTAGE

Percent wound contraction is determined by the subsequent formula:

$$\text{wound contraction in percentage} = \frac{\text{Healed Area}}{\text{Total Wound Area}} \times 100$$

RESULTS AND DISCUSSION

FTIR

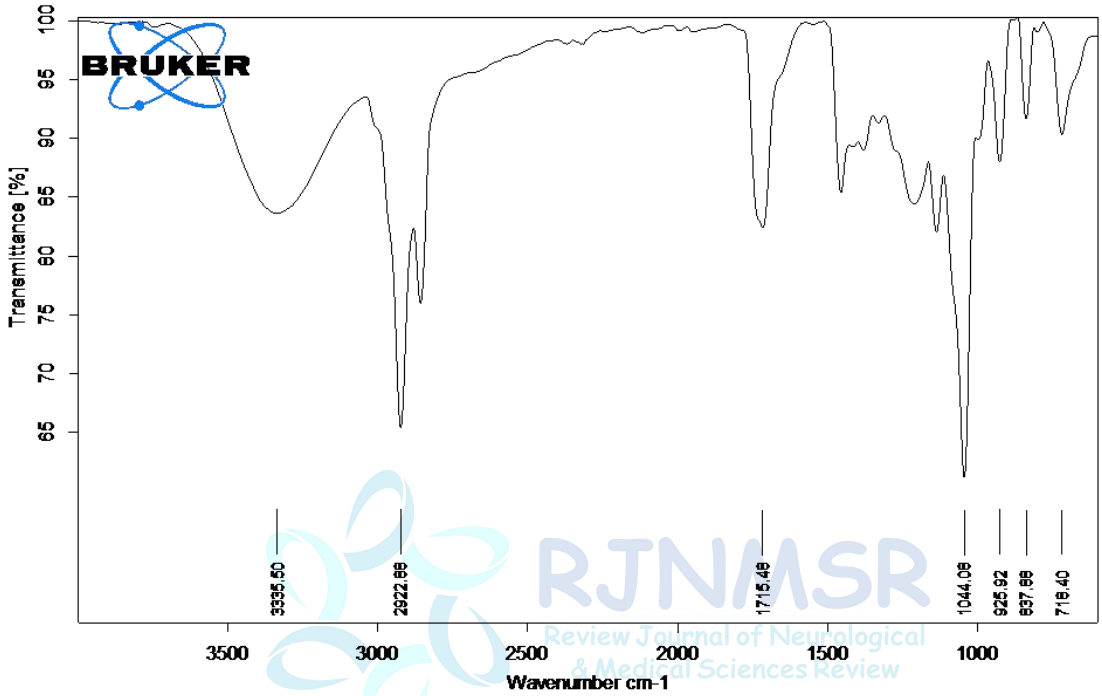

FIGURE 1:FTIR OF NANOETHOSOMES WHICH CONFIRM THE FUNCTIONAL GROUPS VIA FTIR PEAKS

STATISTICAL ANALYSIS

The attained data is analyzed statistically with Graph Pad Prism software and the outcome is equated by the analysis of variance. The set up a significance level at p is lee than 0.05.

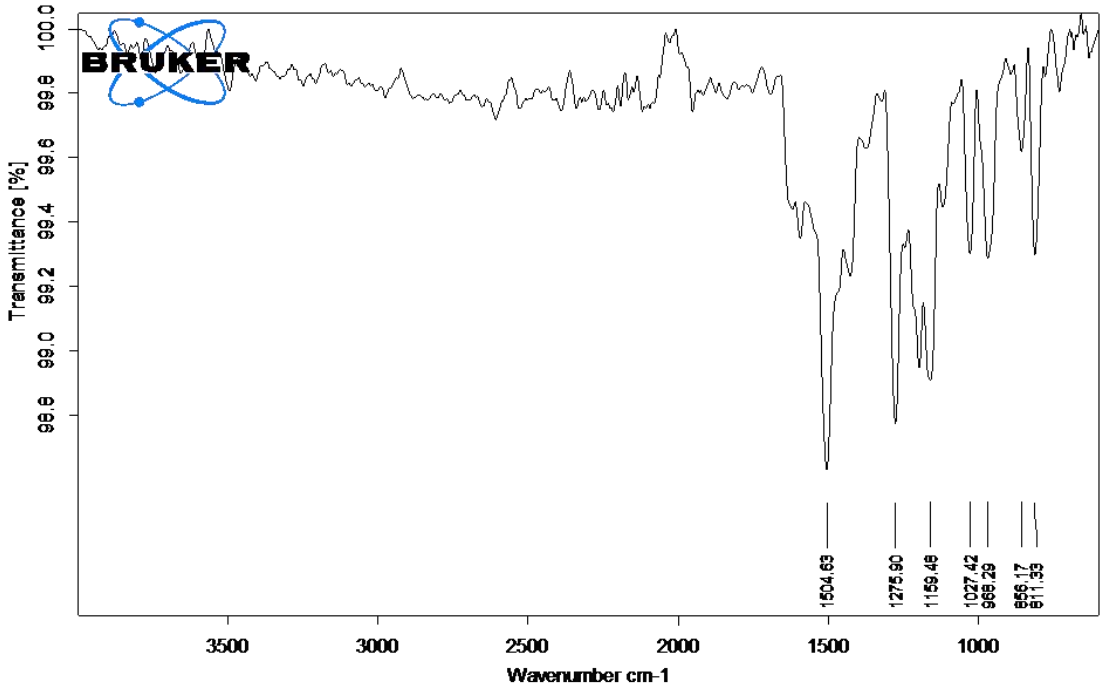

Figure 2:FTIR of curcumin confirms the functional groups present in curcumin via peaks

The observed specific peaks in the FTIR spectra of nanoethosomes with curcumin, ethanol, cholesterol and phospholipids are the consequence of the interactions of their functional groups. O-H stretching vibrations are shown as a broad peak around 3335 cm^{-1}. To be precise, the oscillations are due to effects such as hydrogen bonds present in phospholipids and the hydroxyl groups in ethanol. Cholesterol and phospholipid alkyl chains seem to be responsible for the C-H stretching vibrations for peaks at about 2922 and 2850cm^{-1}. In addition, a clear peak at around 1715 cm^{-1} is observed; the peak corresponds to carbonyl stretching of phospholipid ester from which the lipid bilayer of the nanoethosomes is formed. The band at around 1504 cm^{-1} has been attributed to the carbon-oxygen double bond stretching of conjugated diketone group present in curcumin. There are other major peaks for curcumin at approximately 1275 cm^{-1}, that indicates aromatic C=C stretching and 1159 cm^{-1} indicating C-O stretching respectively. Altogether, these two peaks support that curcumin has been loaded efficiently in the nanoethosomes.

ANTIOXIDANT ACTIVITY

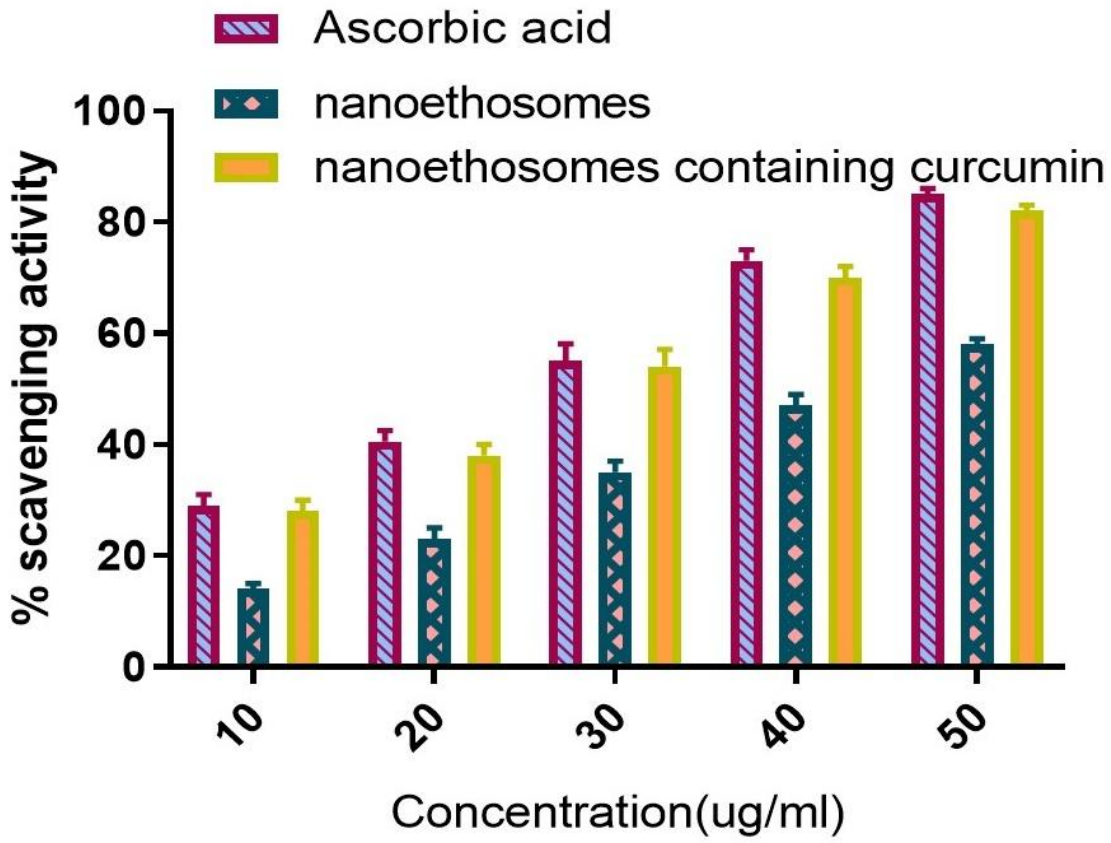

FIGURE 3: Antioxidant activity of nanoethosomes containing curcumin. According to this graph, standard, nanoethosomes and nanoethosomes containing curcumin shows antioxidant activity. nanoethosomes containing curcumin shows best results

Thus, the free radical scavenging method was used for determining the antioxidant activity of ascorbic acid (standard), curcumin-loaded nanoethosomes and nanoethosomes. The outcomes were noteworthy. Some of the experiments were carried out at concentrations of 10, 20 and 50 µg/ml in order to study the potency of these formulations as antioxidant agents at different concentrations. Thus, a direct relationship between ascorbic acid, nanoethosomes, and nanoethosomes having curcumin and the antioxidant activity was observed. The free radical scavenging activities of nanoethosomes were determined as 14 ± 1%, 23 ± 2%, 35± 2%, 47 ± 2%, and 58 ± 1%. In terms of scavenging activities, curcumin loaded nanoethosomes were found to possess 28± 2%, 38± 2%, 54± 3%, 70± 2%, and 82± 1% at concentrations of 10, 20, 30, 40, and 50 µg/ml respectively (Fig.3).

WET AND DRY GRANULATION WEIGHT

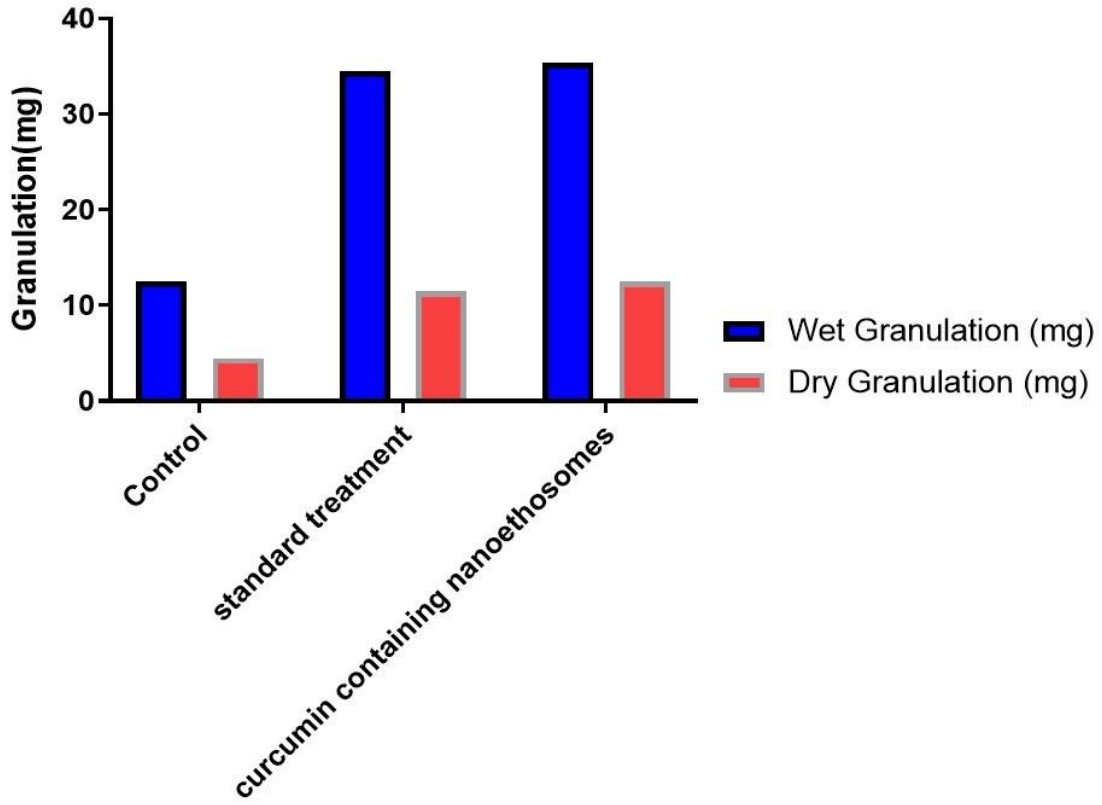

Figure 4:Wet and dry granulation of groups control, standard treatment and nanoethosomes containing curcumin

Granulation tissue is an essential part of the wound healing process and hence, during the course of the experiment, evaluation of healing capacity was performed by comparing curcumin-loaded nanoethosomes, standard treatment, and the control group. After 24 h of drying at 60°C, dry and wet granulation tissue was found increased significantly in the curcumin-loaded nanoethosomal treated group and the values were recorded 35±0. 25 and 12. 48±0. 30.

PERIOD OF EPITHELIALIZATION

TABLE 1 PERIOD OF EPITHELIZATION IN DIABETIC WOUNDS

Group	Epithelialization period (Days)
Control	15± 0.48
curcumin-loaded nanoethosomes	10 ± 0.65 *
standard treatment	12± 0.41*

Table 1 presents the results of the investigates that were made in the study of the research problem and can be concluded that: 1, prove that nanoethosomal-curcumin caused a reduction in epithelialisation time when compared to the control group. The epithelialisation period of the given group treated with curcumin loaded nanoethosomes was significantly small compared to the control group. In addition, the impact of the conventional therapy on epithelialisation was found to be significantly greater as compared to the control group.

PERCENT WOUND CONTRACTION

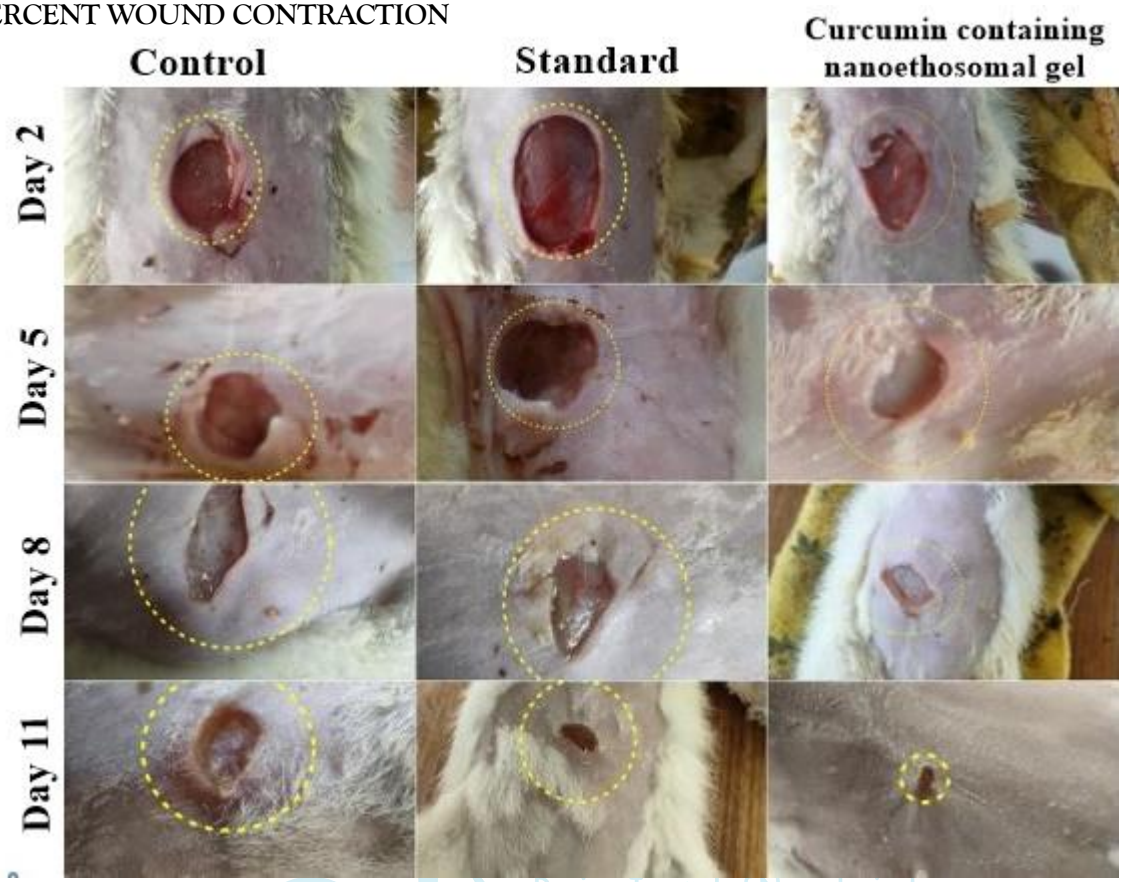

FIGURE 5 WOUND HEALING IN RATS OF GROUPS CONTROL, STANDARD AND NANOETHOSOMES CONTAINING CURCUMIN ON DIFFERENT DAYS

The results demonstrated in Figure 5 prove that by the second day of the experiment the standard treatment and nanoparticles containing curcumin had no effect on the contraction of the wound. P value of 0. 001 shows that on the 5th day, the curcumin containing nanoparticles had a highly significant difference on wound contraction. From this it must be understood that by the fifth day the nanoparticles had fostered contraction and hastened the healing process. Also, it is further evident through significant P value of 0. 001 (Figure 4. 6) proving thereby both treatments supported wound healing. Notably, the eleventh day's consecutive high value of 'sig', the P value was 0. The result indicated in figure 001 is very revealing and confirm that both treatments were exceedingly effective in regards to wound contraction and the time required for the contraction process (Figure 6).

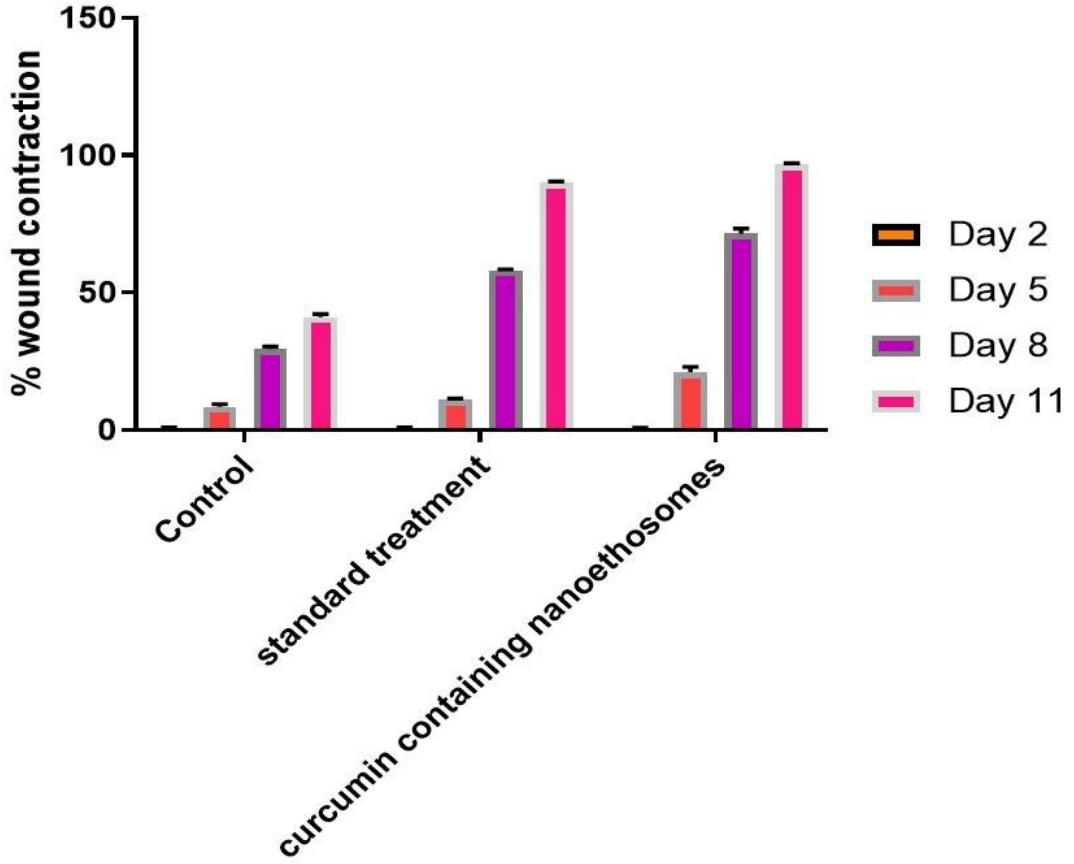

FIGURE 6:GRAPH SHOWS PERCENTAGE WOUND CONTRACTION IN CONTROL, STANDARD TREATMENT AND NANOETHOSOMES CONTAINING CURCUMIN GROUPS

DISCUSSION

The characterization of the nanoethosomes was done using FTIR spectra and the peaks indicated were unique and characteristic of the functional groups of the phospholipids, cholesterol, ethanol and curcumin used in the preparation of the nanoethosomes formulations. The O-H stretching vibrations identified by a broad peak at 3335 cm^{-3} corresponds to hydrogen bonding in phospholipid and ethanol molecules hydroxyl groups. Two small peaks at 2922 and 2850 cm^{-1} can be linked to C-H stretching in regard to the fact that phospholipids and cholesterol may contain alkyl chains. The peak at 1715 cm^{-1} is of great concern because; the function of C=O stretching of the phospholipid ester groups is essential in the s formation of the lipid bilayer in nanoethosomes. The band recorded at 1504 cm^{-1} attributed to C=O stretching in its conjugated diketone group demonstrates the existence of curcumin. Moreover, C=C and C-O stretching vibrations of aromatic rings of curcumin are observed in nanoethosomes at around 1275 cm^{-1} and 1159 cm^{-1}, respectively, demonstrating successful incorporation of curcumin into nanoethosomes (Elsayed et al., 2021). The impact on the antioxidant activity of the phytochemicals presented herein yielded that both ascorbic acid, nanoethosomes as well as curcumin-loaded nanoethosomes possessed enhanced potential for reacting with the free-

radicals in a dose-dependent fashion. The antioxidant activity rose in combination with the concentration enlargement of Berberine from 10 to 50µg/ml. The scavenging activity of nanoethosomes was 14±1% to 58±1%, while the nano ethosomes containing curcumin showed the better activity 28±2% to 82±1% activity. These outcomes suggest that curcumin increases the antioxidant potential of nanoethosomes, making the formulation even better than nanoethosomes more so when used alone (Bin Jardan et al., 2023). The efficiency of curcumin-rigid nanostructured liquid ethosomes in the treatment of wound healing was evaluated based on the level of analysis of granulation tissue compared with control and standard treatment groups. After 24hrs of drying the group that received the treatment with nanoethosomes –curcumin had a notable significant enhanced in granulation tissue wet and dry with means of 35±0. 25 and 12. 48±0. 30, respectively. This indicates that, curcumin-loaded nanoethosomes enhance the granulation process which is key in healing of the wound. Moreover, the epithelialisation period of curcumin-loaded nanoethosomal group was statistically significantly lower than that of the control group suggesting the potential of the formulation in providing an accelerated healing of the wound. It was found that Curcumin-loaded nanoethosomes had epithelialisation time reduced mainly compared to the standard treatment while the variation was prominent (Mekala & Shaheedha). On the second day, there was no favorable effect of the conventional treatment or the curcumin loaded nanoethosomes regarding wound contraction. It can be seen that on the fifth day, wound contraction was influenced by curcumin loaded nanoethosomes (P = 0. 001) and thus, there would be an enhanced healing rate. A P value of 0. 001 shows that there were a significant difference between the effects of standard treatment and curcumin-loaded nanoethosomes on the wound contraction at the end of eighth day. Such a pattern was continued until the eleventh day and the results pointed out the lasting effectiveness of the treatment in promoting the wound healing as well as the contraction of the wound during the course of investigation. It has been observed that curcumin-loaded nanoethosomal formulation exhibited a marked effect on wound contraction, thus establishing them as an effective treatment modality for enhancing the rate of wound healing (Safta et al., 2022).

CONCLUSION

To sum up, this study showed that curcumin-loaded nanoethosomes could be a novel therapeutic strategy for the healing of diabetic wounds. The formulations of nanoethosomal curcumin exhibit enhanced stability, bioavailability, and therapeutic efficacy, making them a promising option for future development and clinical application. Nanoethosomal delivery systems may give patients with chronic wounds and other difficult-to-treat conditions new hope as nanotechnology advances, ultimately improving patient outcomes and quality of life.

REFERENCES

Alsarayreh, A. Z., Oran, S. A., Shakhanbeh, J. M., Khleifat, K. M., Al Qaisi, Y. T., Alfarrayeh, I. I., & Alkaramseh, A. M. (2022). Efficacy of methanolic extracts of some medicinal plants on wound healing in diabetic rats. *Heliyon*, *8*(8).

Baschieri, A., & Amorati, R. (2021). Methods to determine chain-breaking antioxidant activity of nanomaterials beyond DPPH•. A review. *Antioxidants*, *10*(10), 1551.

Bin Jardan, Y. A., Ahad, A., Raish, M., & Al-Jenoobi, F. I. (2023). Preparation and Characterization of Transethosome Formulation for the Enhanced

Delivery of Sinapic Acid. *Pharmaceutics*, *15*(10), 2391.

Burgess, J. L., Wyant, W. A., Abdo Abujamra, B., Kirsner, R. S., & Jozic, I. (2021). Diabetic wound-healing science. *Medicina*, *57*(10), 1072.

Elsayed, M. M., Okda, T. M., Atwa, G. M., Omran, G. A., Abd Elbaky, A. E., & Ramadan, A. E. h. (2021). Design and optimization of orally administered luteolin nanoethosomes to enhance its anti-tumor activity against hepatocellular carcinoma. *Pharmaceutics*, *13*(5), 648.

Karthikeyan, A., Senthil, N., & Min, T. (2020). Nanocurcumin: A promising candidate for therapeutic applications. *Frontiers in Pharmacology*, *11*, 487.

Mekala, K., & Shaheedha, S. EXPLORING THE EFFICACY OF POLYHERBAL NANO-ETHOSOMES IN THE TREATMENT OF BURN WOUNDS.

Nair, R. S., Billa, N., Mooi, L. Y., & Morris, A. P. (2022). Characterization and ex vivo evaluation of curcumin nanoethosomes for melanoma treatment. *Pharmaceutical Development and Technology*, *27*(1), 72-82.

Safta, D., Bogdan, C., & Moldovan, M. (2022). Vesicular Nanocarriers for Phytocompounds in Wound Care: Preparation and Characterization. Pharmaceutics 2022, 14, 991. In: s Note: MDPI stays neutral with regard to jurisdictional claims in published ....