

## Curcumin Nanofibers for Effective Treatment of Diabetic Foot Ulcer: Formulation Development

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### ABSTRACT

Diabetes often causes wounds to heal more slowly and to worsen over time, leading to diabetic wounds or diabetic foot ulcers. With increase in cases of diabetic patients, the number of patients with diabetic wounds is also increasing. In research for the treatment of these wounds, nanofibers and nanogels are observed to be one of the effective ways to cure diabetic wounds. Curcumin was also found to be effective in lowering inflammation and fastening the healing rate. Aim of present research was to formulate curcumin nanofibers with collagen originated from fish. Drug permeation study suggested highest release pattern of F4 nanofiber with maximum amount of collagen content. Further, kinetic studies revealed that n value was less than 0.45 which means diffusion and permeation mechanisms followed. Optimized nanofiber showed antibacterial and antioxidant activity due to presence of curcumin extract. It is anticipated that nanofiber with regulated curcumin release capabilities would be considered as a possible treatment option for diabetic foot ulcers.

**Keywords:** Curcumin extract, antioxidant activity, antibacterial activity, permeation studies, in vivo studies.

### INTRODUCTION

Diabetes mellitus is a major reason behind normal wound recovery impairment. Diabetes is a complicated condition as well as a long-term health issue. The rate of diabetes is rapidly rising globally these days as a result of changes in lifestyle like reduced workout, a high fat meal that leads to weight gain, and ageing population. The feet are the site of the majority of chronic wound concerns in diabetic patients [1,2]. Although other regions may be concerned, the most frequently impacted region is the feet for a variety of causes. Based on certain reports, one in four diabetics will establish diabetic foot ulcers (DFU). These ulcers are uncomfortable blisters that may eventually require surgical removal. Turmeric, a spice which has been seen because of its therapeutic qualities, has gained attention from both the health industry as well as from foodservice industry, as it is the main source of the curcumin. Curcuma longa, an herb with rhizomes, belongs to Zingiberaceae family and is the source of turmeric [3,4]. Turmeric has a curcuminoid content ranging from 2% to 9%, based on location it was cultivated and the characteristics of soil. The term "curcuminoid" refers to a class of substances that includes cyclic curcumin, bis-desmethoxycurcumin and desmethoxycurcumin. The main ingredient among these is curcumin. Curcumin, also known as diferuloyl-methane, is primary polyphenol found in nature. The active ingredient in turmeric, curcumin, is a brilliant orange-yellow, crystalline molecule that is nearly water insoluble at neutral or acidic pH levels. However, it shows solubility in alkaline or strongly acidic solvents like glacial acetic acid as well as organic nonpolar and polar solvents [5].

The most popular technique for isolating curcumin extract has been solvent-extraction accompanied by column chromatographic technique. Various organic polar solvents and non-polar, such as acetone, ethyl acetate and methanol, has been utilized. Ethanol is considered to be the most favoured organic solvent for extraction of curcumin. Curcumin can be isolated from desmethoxycurcumin and bisdemethoxycurcumin (curcuminoid) using column chromatography on silica gel by using solvents such as methanol/chloroform or dichloromethane/acetic acid to produce three distinct fractions. The anti-infectious, anti-inflammatory, and antioxidant properties of curcumin are thought to be responsible for its ability to heal wounds. It affects different phases of wound-healing cycle to speed up recovery. Curcumin has its role in tissue remodelling, granulation, collagen deposition, and tissue creation. Numerous research has demonstrated that topical use of curcumin to wound areas improves epithelial re-generation, stimulates fibroblast proliferation, and boosts vascular density and the wounded area produced more dense, oriented collagen fibres [6-8].

Similar to industrial revolution, nano-technology is considered to be one of the most important scientific and innovative aspect in the twenty-first century. It will significantly impact the words economic system, industrial sectors, manufactured goods, and relaxation in the upcoming future. Nanofibers have diameter of under 100 nm. They belong to a unique class of nanomaterials as they can be arranged into highly porous structural formation that are useful in a wide range of applications, like natural bio-logical tissues [7,8]. To create nanofibers with uniform sizes and morphological characteristics, it is necessary to regulate variables like polymeric concentration and jet stream movement. A wide variety of polymers can be converted into nanofibers using this technique. Web of electrospun nanofibers and the extra-cellular matrix (ECM) are closely related. This similarity is a key benefit of electro-spinning technique because it allows for the imitation of mechanical properties, fibre diameter and high porosity of ECM (Figure 3). Additional advancements in electrospinning are made in order to produce ongoing nanofibers in large quantities. Along with having the ability to create nanofibrous mesh with increased surface area and porosity, this technique also has the benefit of being extremely versatile and is capable of working with a broad range of synthetic as well as natural polymers.<sup>8</sup> However, this method also has drawbacks, including a wide variety of random alignment of nanofibers, fibre thickness and weak mechanical characteristics. In general, electrospinning is a generally reliable and straightforward method for creating nanofibers from a vast range of polymers [9-12].

Present research focused on preparation and evaluation of nanofiber using curcumin extract for diabetic wound by electrospinning method.

## 1. MATERIALS AND METHODS

### *Materials*

Curcumin extract was purchased from Vital Herbs, New Delhi. Collagen was procured from Meneki Global Pvt. Ltd. Pune, Maharashtra, India. Dimethyl acetamide (DMA) and other solvents were purchased from Central Drug House Pvt Ltd, Gujrat, India.

## 2. METHODS

### **Development of nanofibers**

Electrospinning method was used for development of nanofibers. Polymeric solution was obtained by dissolving Cellulose Acetate in acetone and dimethyl acetamide according to the desired formulation design. Curcumin extract along with collagen were mixed (Speed: 800 rpm, Temperature: 40°C). Polymeric solutions were electrospun using electrospinning device (Table 1). Process parameters like potential, flow rate, coating time and distance between drum and collector were 17 kv, 1 ml/hr, 3.0 hrs and 16 cm respectively [13-15].

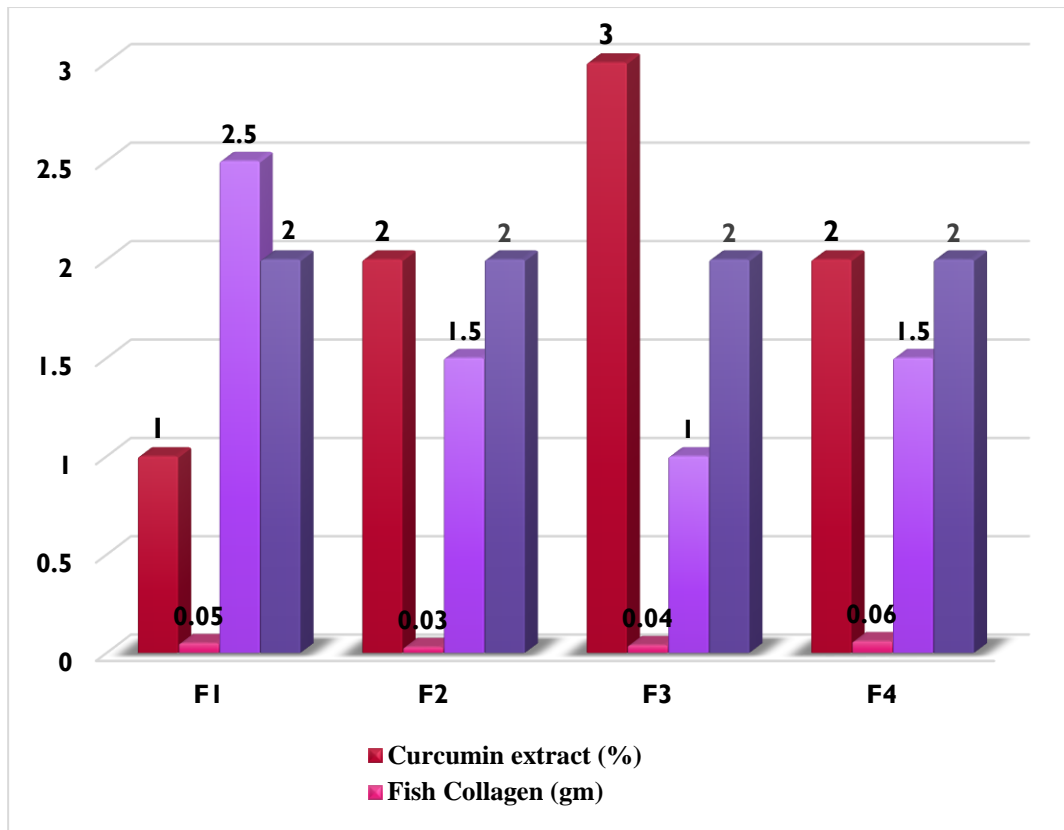


Figure 1: Formulation design of nanofibers (Acetone was added equally in all formulations i.e. 8 ml)

### Permeation Studies

The Franz diffusion cell was used to conduct in vitro permeation investigations. More specifically, the donor and receptor compartments of the cell needed to be separated by a synthetic cellophane membrane [16-18]. After mounting the cellophane membrane on the receptor compartment that was filled with PBS at pH 6.8, a portion of the prepared nanofibers, measuring 1 centimetre square, was put on top of it. Stirring was maintained at 50 rpm at temperature 37°C. Samples of 1 ml were withdrawn at intervals of 1, 2, 3, 4, 5, 6, 7 hours, and drug permeation was analyzed using UV spectroscopy at 210 nm.

### Parameters of Skin Permeation Studies

A graph was created to show how much curcumin penetrated the skin per unit area over time. The volume that crosses the membrane per unit area per unit time is known as the membrane permeation flux [19]. The flux ( $J_{ss}$ ) was found using the X-intercept and the slope of the linear component of the graph using following formula.

$$K_p = J_{ss} / C_o$$

### Mathematical Modelling of Release Kinetics

Permeation kinetics of nanofibers were determined by model dependent parameters i.e., zero order, Higuchi, first order and n value through korsmeyer peppas for mechanism of release from nanofibers [20].

### Morphology

To examine the morphological properties and diameter of electrospun fibers, a Field Emission Scanning Electron Microscope (FESEM, JEOL-JSM 7600F) was used. A voltage of 5 kV was selected for the acceleration [21]. Using an ion sputtering technique, samples were coated with platinum. Using the ImageJ software, fibre sizes were extracted from the SEM pictures.

### Antibacterial Study

Inoculum of gram-positive bacteria (*Staphylococcus aureus*) was standardized to 0.1 mL by adjusting the optical density of the bacterial suspension to match a spectrophotometric absorbance of 0.546 at 630 nm. Curcumin nanofibers that were electrospun had their antibacterial activity standardized at 50 mg [22], whereas the control nanofibers' antibacterial activity was set at 50 mg for the assessment. A temperature of 37°C was maintained during the 24-hour incubation period. The optimized formulations, F1 and F4, were carefully placed on the inoculated plates and incubated for 24 hours at 37°C. The

antibacterial effect was determined by measuring the clear inhibition zone formed around each sample.

#### **DPPH Method for in-vitro antioxidant activity determination**

Ascorbic acid as a standard and 1 ml of various herbal sunscreen concentrations were collected and placed in separate vials. Following a vigorous shake and a 20-minute incubation period at 37°C, 5 mL of DPPH methanolic solution was added [23-25]. A blank of methanol was used to test the absorbance at 516 nm. A control measure was the DPPH absorbance. Calculating the proportion of antiradical action involved using the following formula:

$$\% \text{ Anti - radical activity} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

#### **In vivo study**

**Animal Model:** Streptozotocin-induced diabetic Wistar rats were used, divided into three groups: untreated control, conventional curcumin-treated, and curcumin nanofiber-treated (n = 10 per group) [26].

**Treatment Protocol:** Full-thickness excisional wounds (1 cm diameter) were created on the dorsal surface. Curcumin nanofibers were applied daily for 21 days in the experimental group. Conventional curcumin gel was used for the second group, while the control group received saline. Parameters such as skin irritation, wound contraction, re-epithelialization, granulation tissue formation, Histological changes (collagen deposition, angiogenesis, inflammatory cell count) and inflammatory markers (e.g., IL-6 and TNF- $\alpha$ ) were assessed [27,28].

### **3. RESULTS AND DISCUSSIONS**

#### **Permeation studies**

The release patterns of all nanofibers are illustrated in Figure 2. The F4 nanofiber, which contains 0.06 g of fish collagen, demonstrated the highest release of curcumin extract compared to the others. Fish collagen, being biodegradable, gradually degrades in PBS 6.8, resulting in a controlled release pattern for F4. After 5 hours, the release curve gradually decreases, indicating a controlled release of curcumin through diffusion and nanofiber degradation (Figure 2). In this delivery method, when the nanofibers break down, the medication is consistently released from the nanofiber laden with the extract. The bioactive component's diffusion out of the nanofibers and the polymeric nanofibers' breakdown can be used to gauge the drug's release. Consequently, the formulations exhibited controlled release, progressively delivering the precise dosage of drug. The curcumin flux from the nanofibers was measured to be 12.04 g/cm<sup>2</sup>/hr for F2, 10.30 g/cm<sup>2</sup>/hr for F2, 7.05 g/cm<sup>2</sup>/hr for F3, and 13.82  $\mu$ g/cm<sup>2</sup>/hr for F4.

#### **Model dependent parameters**

Since that the n values were less than 0.45, the data suggested that Fickian diffusion was the primary mechanism of curcumin release from F4 nanofibers. The fish collagen matrix might also undergo erosion, contributing to the diffusion mechanism. Therefore, the release of curcumin is likely influenced by both diffusion and erosion [29].

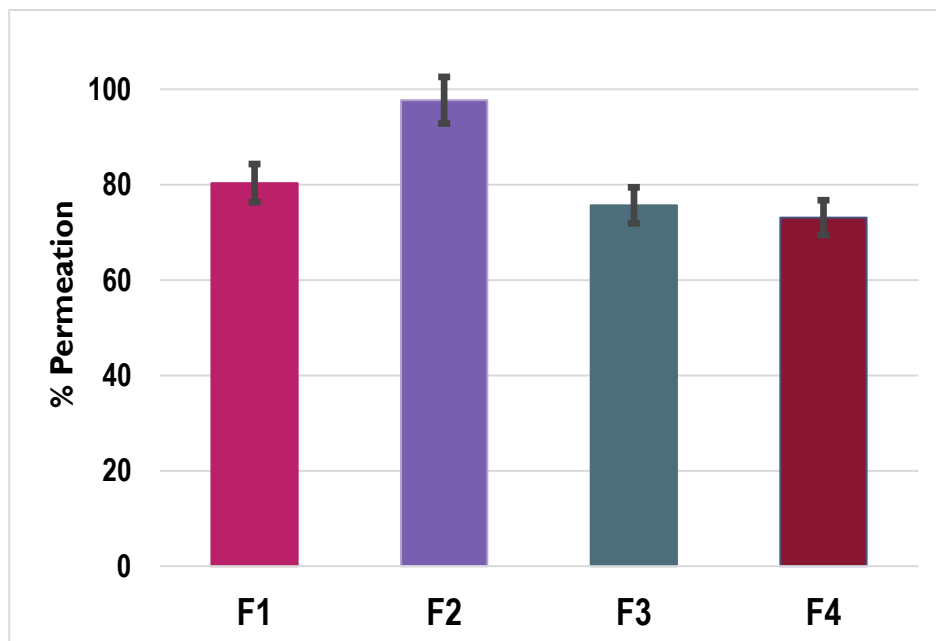
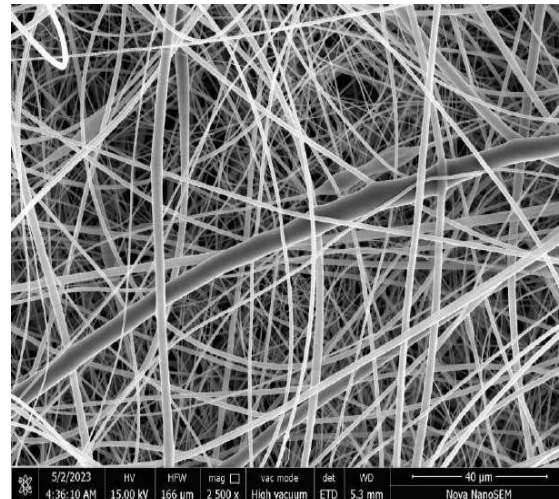


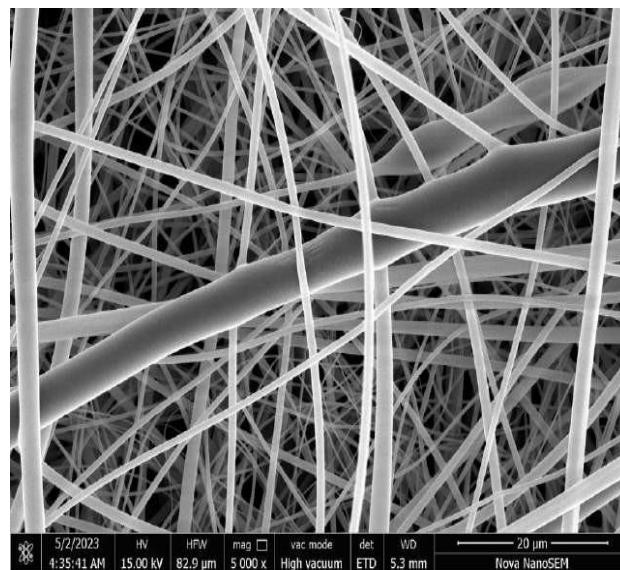
Figure 2: Permeation patterns of nanofibers (F1-F4).

### **Morphology**

The surface morphology of the prepared nanofibers was examined, with images captured at 25.0kx magnification. F2 had a diameter of 79.41 nm, while F4 had a diameter of 130 nm (Figure 3). This suggested that although the probability of bead production reduces, greater polymer concentrations produce nanofibers with bigger diameters and better uniformity. During electrospinning, highly viscous polymer solutions experienced prolonged stress relaxation times, preventing unsuccessful stream throw. Furthermore, increasing the concentration or viscosity of the solution resulted in the production of bigger and more uniform nanofibers. In low-viscosity polymer solutions, surface tension dominated, causing the formation of beaded nanofibers. Jet intensity was impacted by the polymer flow rate inside the syringe, which also had an impact on the diameter and shape of the nanofibers [30].



(A)



(B)

**Figure 3: Surface morphology of nanofibers (A) F2; (B) F4**

### **Antioxidant activity**

Topically applied antioxidant supplements can neutralize reactive oxygen species from both internal and external sources. The stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) produces a violet color shift in ethanol that can be measured at 517 nm [25,31]. When antioxidants react with DPPH, the violet color intensity decreases. In the study, 2 ml of 0.5 millimolar DPPH solution was mixed with nanofibers, and after 30 minutes, the absorbance was measured at 517 nm. The DPPH method was used to determine the anti-radical activity of equally diluted materials, revealing that the activity was concentration-dependent. The antioxidant activity of ascorbic acid in F1, F2, F3, and F4 was found to be 76.45%, 71.23%, 68.87% and 90.56%, respectively. Compared to regular ascorbic acid, F2 and F4 nanofibers exhibited the highest antioxidant activity.

### Antibacterial study

The zone of inhibition produced by the optimized formulations was measured. Compared to the control group, the nanofiber formulations F2 and F4 exhibited antibacterial zones of inhibition against *S. aureus*. This indicates that curcumin is an effective bactericidal agent and could be frequently used as a topical ointment for treating various wounds. Curcumin combats a broad spectrum of bacteria by reversibly inhibiting isoleucyl-transfer RNA, thereby blocking bacterial protein and RNA synthesis [17,31]. F2 demonstrated the strongest activity against *S. aureus* with a zone of inhibition of 1.9 cm, followed by F4 with 0.90 cm. The excellent results in our experiment showed that the formulations are capable of inhibiting the Gram-positive bacterium *S. aureus* and are beneficial for wound healing.

### In vivo studies

The curcumin nanofiber group exhibited significant re-epithelialization and enhanced collagen fiber alignment compared to the control and standard treatment groups. Reduced infiltration of inflammatory cells was observed in the nanofiber group, indicative of an accelerated resolution of inflammation. Significant reductions in malondialdehyde (MDA, a marker of oxidative stress) and pro-inflammatory cytokines (e.g., IL-6, TNF- $\alpha$ ) were observed in the curcumin nanofiber group. Elevated levels of antioxidant enzymes (SOD and catalase) were recorded, reflecting enhanced oxidative defense. The use of curcumin-loaded nanofibers showed a marked improvement in wound healing metrics, with superior wound contraction rates, increased collagen deposition, and angiogenesis compared to standard treatments.

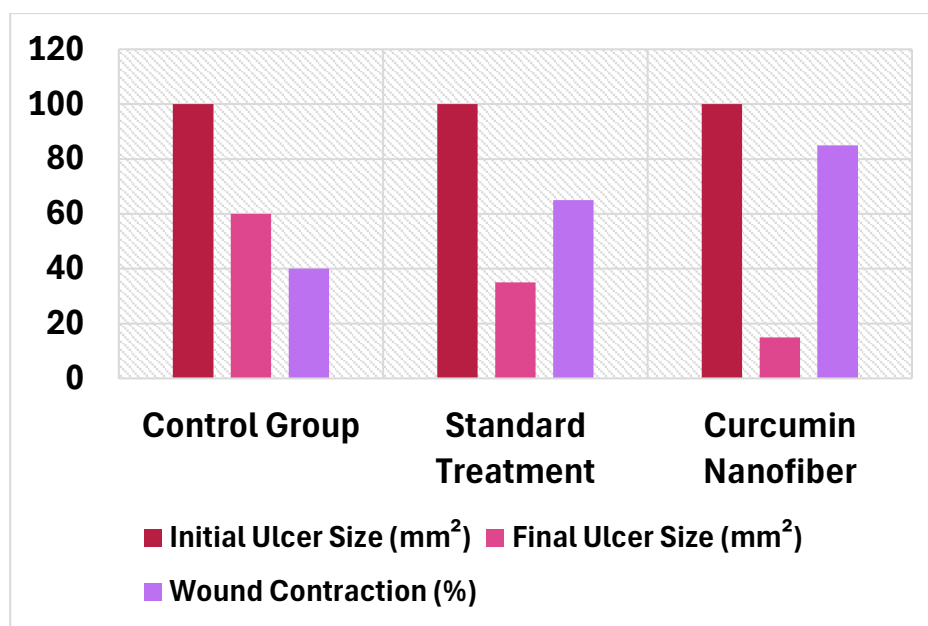


Figure 4. Wound contraction and healing metrics

The nanofiber's sustained release of curcumin likely enhanced its therapeutic effects by maintaining optimal local concentrations. These findings suggest that curcumin nanofiber dressings could offer a promising alternative for managing DFUs, leveraging the multifunctional benefits of curcumin in a bioavailable format. The group treated with curcumin nanofibers showed significantly improved wound closure compared to both the control and conventional curcumin-treated groups. Histological analysis indicated enhanced epithelialization and collagen deposition, pointing to accelerated tissue regeneration. Furthermore, the nanofiber group demonstrated a notable decrease in pro-inflammatory cytokines (TNF- $\alpha$  and IL-6) and an increase in antioxidant enzyme activity (SOD) (Figure 4). These results underscore the potential of curcumin nanofibers to address the complex challenges of diabetic foot ulcer (DFU) healing through sustained drug delivery and amplified therapeutic effects of curcumin. Data were analyzed using ANOVA followed by Tukey's post hoc test. The curcumin nanofiber group showed significantly better outcomes compared to the other groups ( $p < 0.05$ ). The irritation score was minimal, indicating that curcumin-loaded nanofiber is non-irritant.

Table 1. Observation parameters for wound healing in diabetic rats ( $\pm$ SD)

Parameter	Control Group	Standard Treatment	Curcumin Nanofiber
Initial Ulcer Size (mm <sup>2</sup> )	100 $\pm$ 2.5	100 $\pm$ 2.3	100 $\pm$ 2.4

Final Ulcer Size (mm <sup>2</sup> )	60 ± 3.0	35 ± 2.8	15 ± 1.2
Wound Contraction (%)	40 ± 3.2	65 ± 2.5	85 ± 1.8
Collagen Deposition (score)	2.5 ± 0.4	3.8 ± 0.3	4.5 ± 0.2
Angiogenesis (score)	2.1 ± 0.3	3.5 ± 0.4	4.2 ± 0.3
Inflammatory Cells (count)	35 ± 5.2	25 ± 3.6	15 ± 2.4
Re-epithelialization (%)	45.5 ± 4.1	63.4 ± 3.9	90.2 ± 3.2
Granulation Tissue Thickness	1.2 ± 0.2 mm	1.8 ± 0.3 mm	2.6 ± 0.2 mm
IL-6 Levels (pg/mL)	85.4 ± 5.6	68.3 ± 4.7	42.1 ± 3.3
TNF-α Levels (pg/mL)	120.3 ± 7.1	92.4 ± 6.3	51.6 ± 4.5
SOD Activity (U/mg protein)	2.4 ± 0.3	3.1 ± 0.4	4.5 ± 0.6
Draize Irritation Score (0–4)	-	0.3 ± 0.2	0.2 ± 0.1
Erythema Score (Mean ± SD)	0.3 ± 0.2	0.2 ± 0.1	0
Edema Score (Mean ± SD)	0.5 ± 0.1	0.1 ± 0.1	0

#### 4. CONCLUSION

Based on various characteristics nanofibers depict immediate effect as wound dressing and excellent drug delivery system, and show great antibiotics, analgesics. These activities of nanofibers help them in healing process of wound, decreasing inflammation and infection. The most important feature of nanofibers is that they mimic extracellular matrix (ECM) and act like a second skin. They promote proliferation and angiogenesis. The research on drug penetration showed that nanofibers might deliver as much as 90% of the medicine, which is beneficial for long term release. The sustained release of curcumin from the nanofibers likely contributed to these therapeutic effects by maintaining optimal local concentrations. These results indicate that curcumin nanofiber dressings could serve as a promising alternative for managing diabetic foot ulcers (DFUs), harnessing the multifunctional benefits of curcumin in a bioavailable form. In summary, the nanofiber and nanogel showed promising potential in wound healing process.

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