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Combined Use of Photobiomodulation and Curcumin-Loaded Iron Oxide Nanoparticles Significantly Improved Wound Healing in Diabetic Rats Compared to Either Treatment Alone

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Abstract

Introduction: Here, we assess the therapeutic effects of photobiomodulation (PBM) and curcumin (CUR)-loaded superparamagnetic iron oxide nanoparticles (SPIONs), alone or together, on the maturation step of a type 1 diabetes (DM1) rat wound model.

Methods: Full-thickness wounds were inflicted in 36 rats with diabetes mellitus (DM) induced by the administration of streptozotocin (STZ). The rats were randomly allocated to four groups. Group one was untreated (control); group two received CUR; group 3 received PBM (890 nm, 80 Hz, 0.2 J/cm^2); group 4 received a combination of PBM plus CUR. On days 0, 4, 7, and 15, we measured microbial flora, wound closure fraction, tensile strength, and stereological analysis.

Results: All treatment groups showed a substantial escalation in the wound closure rate, a substantial reduction in the count of methicillin-resistant *Staphylococcus aureus* (MRSA), a substantial improvement in wound strength, a substantially improvement in stereological parameters compared to the control group, however, the PBM+CUR group was superior to the other treatment groups (all, $P \leq 0.05$).

Conclusion: All treatment groups showed significantly improved wound healing in the DM1 rat model. However, the PBM+CUR group was superior to the other treatment groups and the control group in terms of wound strength and stereological parameters.

Keywords: Wound healing; Diabetes mellitus; Photobiomodulation; Low level laser therapy; Magnetic iron oxide nanoparticles.

Introduction

Diabetes mellitus (DM) is categorized by a sustained increase in blood glucose caused by inadequate insulin generation, decreased insulin sensitivity generation, or a combination of both (inadequate insulin generation, decreased insulin sensitivity generation).[1] The Centers for Disease Control and Prevention (CDC) reported that 10.5% of the US population had DM in 2017. More than 1 in 3 (34.5%) US adults have prediabetes.[2] DM is defined as fasting blood glucose levels of 126 mg/dL or higher. However, more than 84% of prediabetic individuals are unaware of their condition. Prediabetes has an increased risk of the development of type 2 DM (DM2), cardiac disease, and stroke.[2]

Prolonged high blood glucose levels can cause peripheral neuropathy, atherosclerosis, increased risk of infections, and delayed healing of skin wounds. Diabetics are particularly susceptible to foot problems, neuropathy,

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ischemia, and infected skin wounds, all of which impair the quality of life and have a risk of amputation.[3] Wound healing (restoration) is an active physiological process that consists of the following steps: hemostasis, inflammation, cell recruitment, and maturation. Many different cell types, extracellular elements, and cytokines are involved in wound restoration. By interfering with one or more of the above-mentioned steps, DM decreases the ability of the body to repair injured skin.[4] Diabetic foot ulcers (DFUs) are common and are a major medical problem, resulting in substantial pain and frequent recurrence, and they have an increased risk of amputation or death, along with high medical costs.[5] The average time for DFUs to repair without surgery is about three months, and they often lead to amputation.[6] About 26.5% of patients with DM are expected to be diagnosed with a DFU.[7] Treatment of DFUs accounts for about 33% of the entire cost of diabetic treatment, which was US$ 176 billion in 2012.[8] Despite these costs, around one-fifth of patients with DM still have unhealed DFUs at 12 months.[9]

Regular treatment of DFU includes debridement, bandaging, pressure offloading, blood vessel bypass, control of infection, and reduction of blood glucose levels.[10] However, there is a potential for improved treatment of DFUs,[11] and further investigations are required to discover new approaches to healing DFUs.[12]

During last few years, nanoparticles (NPs) have emerged as important platforms to treat skin wounds. Silver, gold, and copper NPs, as well as titanium and zinc oxide NPs, have shown potential therapeutic effects on wound healing. Due to their specific characteristics, NPs such as nanocapsules, polymersomes, solid lipid NPs, and polymeric nano complexes are ideal vehicles to improve the effectiveness of drugs (antibiotics, growth factors, etc) aimed at wound healing. On the other hand, if active excipients such as curcumin, hyaluronate, or chitosan are added during the formulation, the nanomedicine could significantly improve its potential. In addition, the inclusion of NPs in different pharmaceutical materials may enhance the beneficial effects of the formulations and allow for achieving better dose control.[13]

Curcuma longa is the source of turmeric, which is a therapeutic herb and spice known worldwide for its dietary and medical benefits. Its major component is curcumin, which has been used to treat numerous illnesses, including infections, DM, and wounds. Curcumin can decrease prolonged inflammation via its antioxidant and anti-inflammatory properties. However, its poor aqueous solubility, sub-optimal tissue accumulation, and rapid metabolism prevent the systemic use of curcumin for wound restoration. Recently, numerous formulations of curcumin, including hydrogels and nanopreparations, have been investigated for the direct delivery of curcumin to the injured site. Nanoformulations are an evolving field, and they have numerous uses in wound restoration to deliver active compounds. Researchers have studied various metallic or mineral NPs and reported their benefits in wound restoration.[14] Nanoscale structures have been used to improve wound restoration at various steps of the healing process. Drugs and medications can be formulated at the nanoscale level, and they can either act on their own or be transported by loading onto nanomaterials.[15] Superparamagnetic iron oxide nanoparticles (SPIONs), such as Fe_3O_4, are promising for numerous clinical applications.[16] SPIONs are candidates for effective nano-drug delivery vehicles because they are harmless, their surface can be functionalized, and they can be directed by an exterior magnetic field.[17]

Recently, several studies have examined the effects of curcumin nanotechnology products on cells and tissues in in vitro and preclinical studies regarding the wound repair process action. Kamar et al reported that while treating diabetic skin injuries with CUR + hydrogel (HG) enhanced the therapeutic process moderately, the application of topical CUR + HG + NPs exhibited a significant acceleration of the healing process.[18] Karri et al organized a new nanohybrid scaffold by incorporating CUR in chitosan NPs (CSNPs) to extend solubility and strength and then by impregnating the prepared CUR + CSNPs into a collagen scaffold to increase its efficiency in restoring damaged tissues. Subsequently, Karri et al found that chitosan, together with collagen and CUR, is a talented approach for achieving a better curing ability.[19] In another study conducted by Li et al, the self-assembly of CUR was first performed to expand its bioavailability. CUR was encapsulated in gelatin microspheres to respond to matrix metalloproteinases (MMPs) that are commonly present in DFUs circumstances. The researchers demonstrated that the well-established CNP delivery method can significantly improve wound healing in diabetic wounds.[20] In another study, Liu et al. created curcumin-loaded chitosan nanoparticles (CUR + CS + NPs). They found that CU + CS + NPs could efficiently improve angiogenesis and reduce macrophage-mediated inflammation, thereby promoting diabetic injury repair in models of streptozotocin (STZ)-induced DM.[21] Taghavifar et al assessed the wound repair action of CNPs in diabetic injuries infected with MRSA sensitized with human α-lactalbumin made lethal to tumor cells (HAMLET). Laboratory tests showed that outcomes were greater in the MRSA + CNP + HAMLET group than in the control, CNP, or HAMLET groups. Taghavifar et al concluded that CNP-enhanced diabetic ulcers infected with MRSA were prepared with HAMLET.[22]

Naserzadeh et al designed a preparation of curcumin-loaded SPION NPs (CUR), intending to produce a novel treatment for schizophrenia. They concluded that the CUR displayed effective anti-neurotoxicity activity in the cerebellum of schizophrenic rats. Naserzadeh et

al suggested that this approach could be expanded to include other translational and medical applications.[23] Subsequently, the Bayat group examined the combination and/or single effect of photobiomodulation (PBM) plus CUR in a rodent model of cutaneous wounds. They concluded that CUR, PBM, and PBM + CUR hastened skin wound repair by increasing wound contraction, improving the biomechanical parameters of the repaired tissue, and substantially reducing contamination with *Staphylococcus aureus*. They reported that the PBM + CUR group was significantly better in comparison with the individual PBM and CUR groups.[24]

A review by Oyebode et al in 2021 concluded that PBM is a potentially beneficial modality for hastening wound restoration, relieving pain, and decreasing inflammation by the modification of various biological processes.[25] The results of preclinical studies have suggested that the most successful treatment of wounds in DM models can be attained through the synergistic effect of two separate modalities.[26-28] It has recently been reported that a combination of PBM plus stem cells was more effective than either PBM or stem cells alone in the stimulation of cutaneous wound restoration and moderating the inflammatory response in animals with type one DM (DM1)[27,28] or DM2.[26]

In the present study, we assessed the therapeutic effects of PBM and CUR, alone and in combination, on the maturation step of a DM1 wound model in rats.

Materials and Methods

Animals and Study Design

We used 36 adult male Wistar rats in this study. The rats were treated ethically and maintained in standard animal housing.

After the induction of DM1 (see below) and wounding, the rats were arbitrarily allocated to the following four groups (Gs) (n = 6 per group). Group1 rats were untreated DM1 controls; Group2 received CUR only; Group3 received PBM only; Group 4 received a combination of PBM and CUR (PBM + CUR). We conducted the microbial evaluation and assessment of the wound area on days 0, 4, 7, and 15 after the treatment. All of the rats were euthanized on day 15 by the administration of CO_2, and tissue samples were obtained for tensiometry and histological examinations.

Induction of DM1

Each rat received an injection (IP.) of 40 mg/kg STZ to induce DM1. The rats that had blood glucose levels above 250 mg/kg were reflected to have DM1. All STZ-injected rats were preserved for 30 days for confirmation of DM1 before the experiments.

Wounding

While the rats were unconscious and under sterile settings, a 1.5-cm full-thickness circular wound on the back of each rat was generated. To reduce skin contraction, we secured silicone rings with silk sutures around each wound (Figure 1).[28]

Superparamagnetic Fe3O4 NPs Creation and Curcumin Loading

Curcumin was loaded into the modified NPs at an increased temperature, as stated by a formerly published protocol.[24] Briefly, curcumin was heated in acetone and progressively added to the NP dispersion in purified water. The curcumin was encapsulated into the NPs as previously reported.[24,29] For the evaluation of the contents of Fe_2O_3 NPs in the skin, they were measured 24 and 72 hours post-exposure to the NPs. A transmission electron microscope (TEM) examination was performed on curcumin NPs. The NPs had an average diameter of 5 nm (Figure 2). Complete information on the creation of NPs and curcumin loading, as cytotoxicity tests of NPs *in vitro* and *in vivo*, were provided in Supplementary file 1.

Administration of CUR to Rats

A standard dispersion was prepared by adding 5.4 mg CUR to 1×10^5 µL distilled water. Next, 100 µL of the standard dispersion was added to 140 µL distilled water (total: 240 µL) and divided equally into eight parts, and each part was injected intradermally at a distance of 5 mm adjacent to the wound margin, at eight injection sites (Figure 1). Rats in groups 2 and 4 received CUR on days 0, 4, 7, and 10.[24]

Photobiomodulation

The wounds and adjacent skin areas of the rats in groups 3 and 4 received PBM (890 nm, 0.2 J/cm^2, 80 Hz, Table 1) immediately after wounding, while the rats were sedated. Thereafter, PBM at the same dose was administered 6 days per week for 15 days (Figure 1).

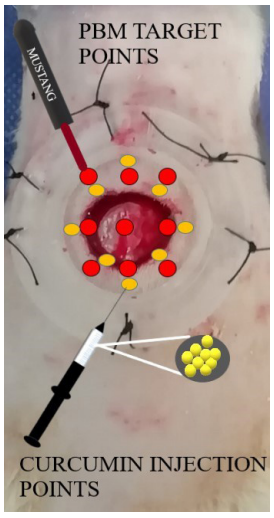

Figure 1. The Wound Location, Silicon Ring Around the Wound, Injection Sites for the Curcumin-Loaded Nanoparticle, and Targeted Laser Points

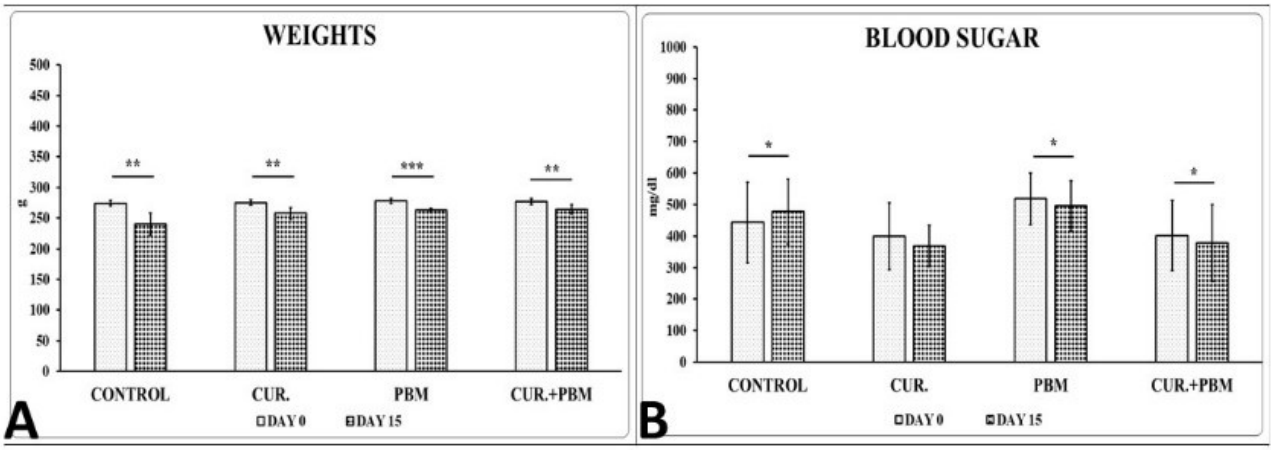

Figure 2. Transmission Electron Microscope Image of Superparamagnetic Iron Oxide (Fe_3O_4) Nanoparticles (CUR)

Table 1. Complete Parameters for *In Vivo* Photobiomodulation

Parameter	Dose and unit
Peak power	75 W
Average power	0.001 W
Power density	0.001 W/cm^2
Wavelength	890 ± 10 nm
Pulse frequency	80 Hz
Duration of pulse	180 ns
Spot size	1 cm^2
Diameter of the spot	1.12 cm
Time of each exposure	200 s
Number of exposures per session	9
Energy density of one exposure	0.2 J/cm^2
Total energy per session	1.8 J
Total energy of all sessions	28.8 J
Model	MUSTANG 2000, Technica Co., Russia L07

Examination of Wound Closure and Completed Wound Contraction

A digital camera was used to capture the wound area on days 0, 4, 7, and 15, and the surface area was calculated by means of the ImageJ program (NIH). The wound closure fraction was computed on each day:

Wound closure fraction = [(wound region on day 0 – wound region on day X)/ wound region on day 0] × 100%.[28]

The healing of the wounds was recorded by careful daily observation of the wounds until day 15.[30]

Microbiological Analysis

Swabs were used to take samples from the wounds on days 0, 7, and 15 for routine microbiological assessments to identify S. aureus and S. epidermidis. The number of microbes per wound was counted and recorded as colony-forming units (CFUs).[24]

Wound Strength

One 5 × 50 mm section from each wound was excised from each rat on day 15. The tissue was placed in a material tensile testing machine (SANTAM, Iran), and the speed of the mobile clamp was set at 0.166 mm/s. The stress-strain curve, bending stiffness (MPa), and stress high load (N/cm^2) of each sample were calculated by computer assessment software.

Histological and Stereological Analysis

On day 16, the samples were extracted from wound tissue and adjacent skin, fixed in formalin saline, and prepared for light microscopic examination. Ten sections (5μ) were stained with a hematoxylin and eosin (H&E) kit, and the numbers of neutrophils, macrophages, fibroblasts, and blood vessels were evaluated by a trained observer fashion blindly by the physical dissector method.[26] Histologic slides were taken from all studied groups.

$Nv = \Sigma Q / (h \times a/f \times \Sigma p)$

Where Nv = numerical density; ΣQ = number of cells; h = height of the dissector; a/f = counting frame area; and ΣP = total number of counting frames.

$N\ (total\ of\ cells\ in\ each\ section) = Nv \times V$

Where V = final total volume

$Number\ of\ blood\ vessels = 2\Sigma Q / (\Sigma P \times a/f)$

Where 2ΣQ = the total number of vessels per rat.[27]

Statistical Analysis

The t-test, one-way analysis of variance (ANOVA), and least significant difference (LSD) tests were used for values that had a normal distribution. The chi-square test was used to compare completely closed wounds among the groups. A P value of $< .05$ was considered statistically significant.

Results

Clinical Observations

We observed that all of the rats had substantial increases in their blood glucose levels and remarkable decreases in weight confirming the induction of DM (Figure 3). The t-test exhibited that rats from the PBM groups had substantial decreases in blood glucose levels on day 15 compared to the day of surgery (day 0). Nevertheless, the values were still within the accepted range for DM1. Blood glucose values in the normal healthy rats were 117.1 ± 2 mg/dL.

Biodistribution of CUR

The biodistribution of CUR is presented in Table 2. Injured skin samples, exposed to curcumin NPs for 24 ($P=0.000$) and 72 hours($P=0.000$), showed a better biodistribution than the healthy skin.

Wound Closure Fraction and Completely Closed Wounds

At both time points, treatment regimens (groups 2, 3, and 4) had improvements in wound closure fraction compared to the control group. However, consistent with the LSD test, the results of the PBM groups (3 and 4) were superior to the CUR group on day 7. The PBM + CUR and PBM groups showed a substantial escalation in wound closure fraction compared to the control group ($P=0.000$, and $P=0.001$) and CUR ($P=0.045$, $P=0.01$) group on day 8. The PBM + CUR, PBM, and CUR groups showed a substantial escalation in wound closure fraction compared to the control group ($P=0.027$, $P=0.030$, $P=0.047$) on day 15 (Figure 4). The PBM + CUR and PBM groups had a significantly higher number of completely healed wounds compared to the CUR and control groups (Chi-square, $P=0.038$). Table 3 shows the mean and standard deviation of entire wound closure fractions of studied groups on days 4, 7, and 15.

Microbiological Analysis

All treatment groups indicated substantial reductions in CFU compared to the control group. The PBM + CUR, PBM, and CUR groups had significant decreases in *S. epidermidis* CFU compared to the control group on day 8 ($P=0.000$, $P=0.028$, $P=0.015$). The CFUs in the PBM + CUR group were significantly lower compared to those in the PBM and CUR groups (all, $P=0.002$). The PBM + CUR and PBM groups had significantly fewer *S. epidermidis* CFUs compared to the control group (both, $P=0.000$) and CUR group ($P=0.000$, $P=0.007$). All treatment regimens had substantial reductions in *S. aureus* CFU compared to the control group on days 8 and 15 (all, $P=0.000$) (Figure 5).

Tensiometric Examinations

All treatment groups (2, 3, and 4) showed a substantial improvement in wound strength compared to the control group; however, the PBM + CUR group was superior to the other treatment groups. The CUR + PBM, PBM, and CUR groups increased bending stiffness compared to the control group ($P=0.000$, $P=0.011$, $P=0.004$). The CUR + PBM group was better than the PBM and CUR groups (both, $P=0.000$). The CUR + PBM, PBM, and CUR treatments significantly increased the stress high load compared to the control group ($P=0.000$, $P=0.032$, $P=0.005$). The CUR + PBM group was superior to the PBM and CUR groups (both, $P=0.000$) (Figure 6).

Histological and Stereological Parameters

Figure 7 shows hematoxylin and eosin-stained slides of all studied groups on day 16. All treatment groups substantially improved stereological parameters

Table 2. Mean ± SD of Superparamagnetic Iron Oxide (Fe3O4) Nanoparticles (CUR, ng/g Fresh Tissue) in the Healthy and Injured Skin at 24 and 72 hours After Exposure to CUR, Using the One-Way ANOVA and LSD Tests

Group	Time Points	
	24 h	72 h
Healthy skin	1.15 ± 0.06	1.2 ± 0.03
Injured skin	2.2 ± 0.04[A]	3.2 ± 0.04[A]

A: significant difference of injured skin groups with healthy skin ($P=0.000$).

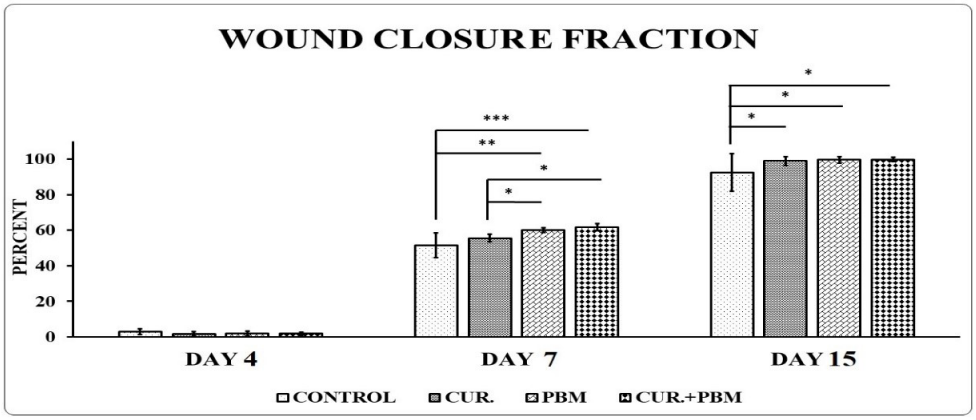

Figure 3. Mean ± SD of Weights of the Rats (A) and Blood Sugar Levels (B) on Days 0 and 15 in the Groups (t-test). *$P<0.05$, ** $P<0.01$, *** $P<0.001$

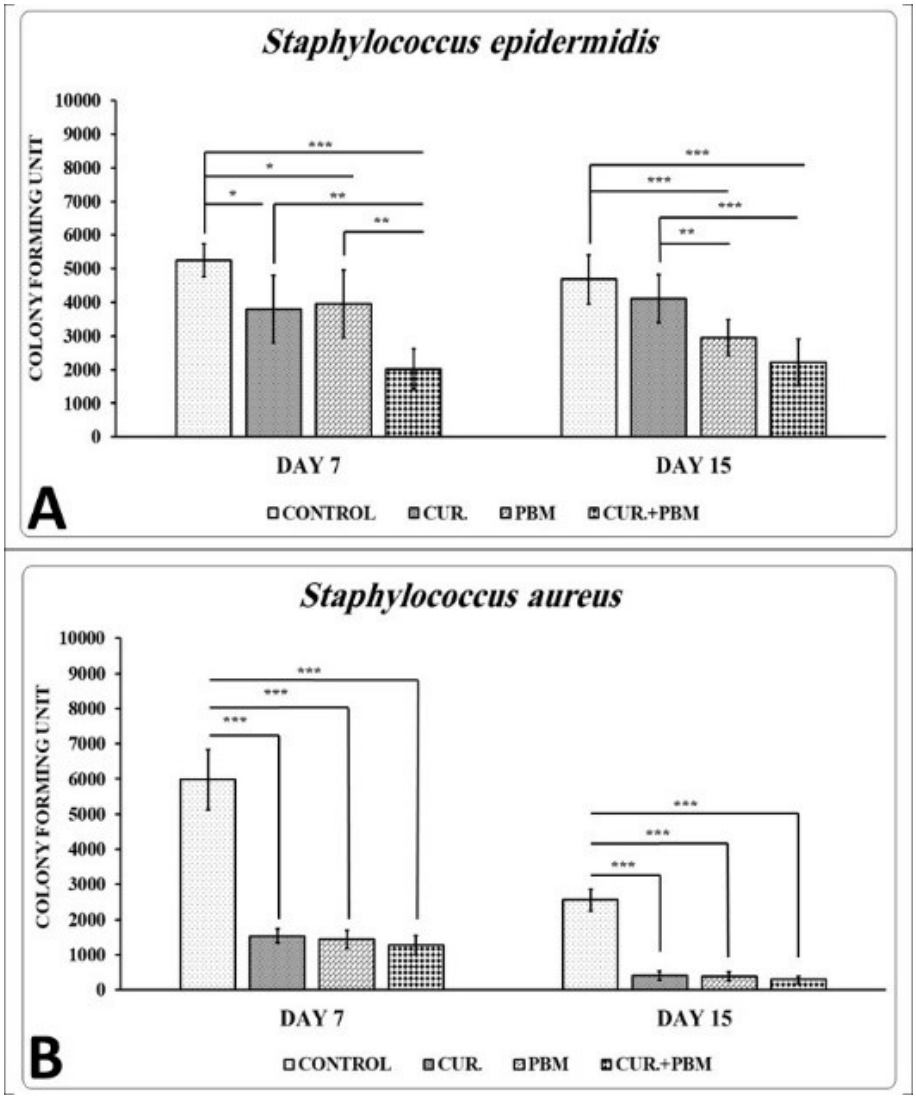

Figure 4. Mean ± SD of Wound Closure Fraction on Days 4, 7, and 15 According to the One-Way Analysis of Variance (ANOVA) and the Least Significant Difference (LSD) Tests. *$P<0.05$, ** $P<0.01$, *** $P<0.001$

Table 3. Mean and Standard deviation of Entire Wound Closure Fractions of the Studied Groups on days 4, 7, and 15

Groups		Wound Closure Fraction, 4	Wound Closure Fraction, 8	Wound Closure Fraction, 15
Control	Mean	3.0658	51.5404	92.4257
	Std. Deviation	1.72885	6.80552	3.06085
CUR	Mean	1.6940	55.6064	99.0267
	Std. Deviation	1.32258	2.19957	1.51295
PBM	Mean	1.9800	60.2348	99.6784
	Std. Deviation	1.31346	1.33162	.78785
CUR + PBM	Mean	1.9583	61.7514	99.8830
	Std. Deviation	.83552	1.86450	.28649

compared to the control group; however, the PBM + CUR group was superior to the other treatment groups (Figure 8).

All treatment groups showed substantial decreases in inflammatory cells (neutrophils and macrophages), individually and together (all, $P=0.000$), compared to control group. The PBM + CUR group showed significant decreases compared to the CUR ($P=0.000$) and PBM ($P=0.003$) groups. All of the treatment groups showed significant increases in fibroblast numbers compared to the control group (all, $P=0.000$). The PBM + CUR group showed significant increases compared to the CUR ($P=0.012$) and PBM ($P=0.041$) groups. All treatment groups showed significant increases in the blood vessel number compared to the control group (all, $P=0.000$), and the PBM + CUR group showed significant increases compared to the CUR group ($P=0.002$) (Figure 8).

Discussion

Some probes have displayed the beneficial effects of PBM on glucose metabolism in hyperglycemic and diabetic subjects. Sene-Fiorese et al conducted a study on females with obesity. They observed that five months

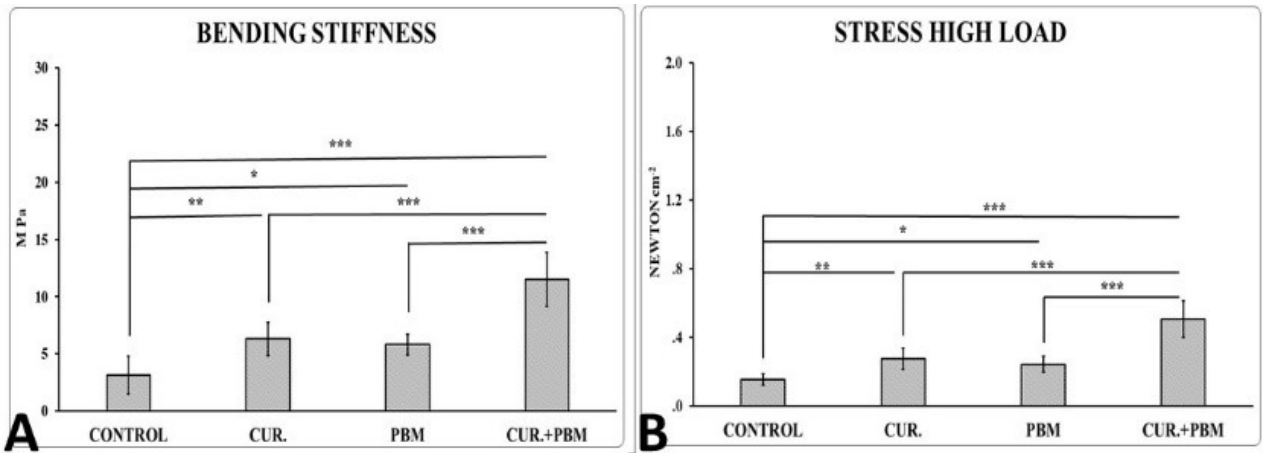

Figure 5. Mean ± SD of Colony-Forming Units (CFUs) of *Staphylococcus epidermidis* (A) and *S. aureus* (B) on Days 8 and 15 According to the ANOVA and LSD Tests. *$P<0.05$, ** $P<0.01$, *** $P<0.001$

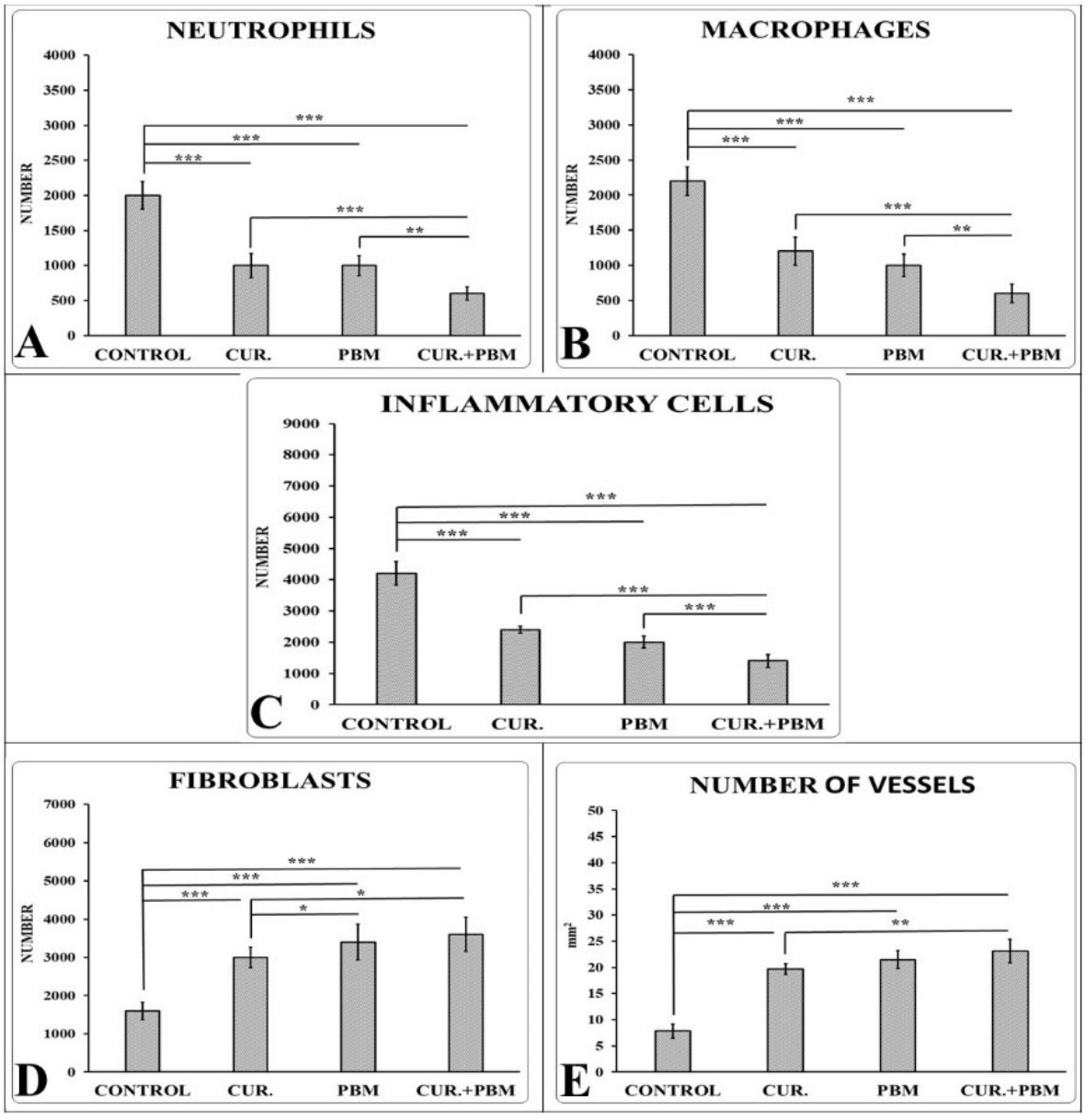

Figure 6. Mean ± SD Bending Stiffness (A) and Stress High Load (B) on Day 15 According to the ANOVA and LSD Tests. *$P<0.05$, ** $P<0.01$, *** $P<0.001$

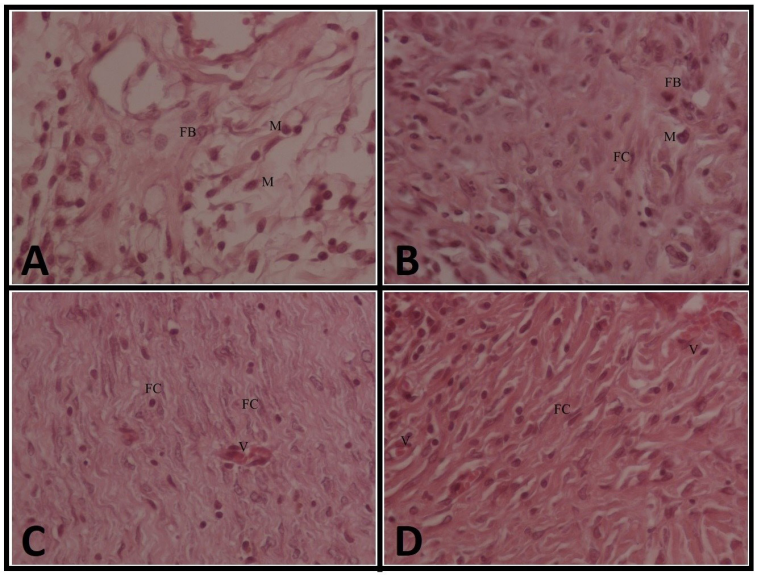

Figure 7. Hematoxylin and Eosin Stained Slides of All Studied Groups on Day 15. Magnification 400×. Abbreviations: M, macrophage; FB, fibroblast; FC, fibrocyte

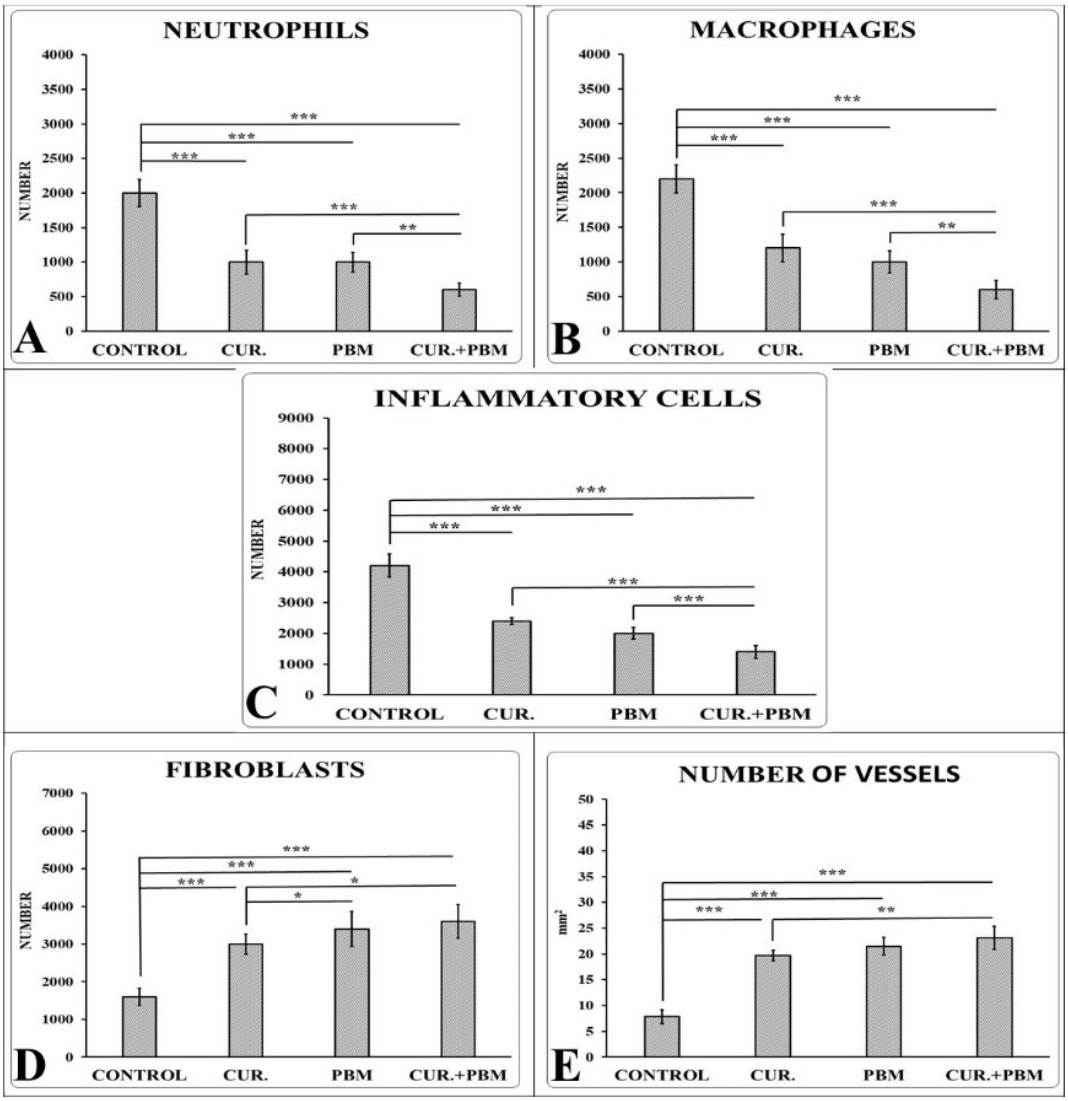

Figure 8. The Numbers of Neutrophils (A), Macrophages (B), Inflammatory Cells (C), Fibroblasts (D), and Blood Vessels (E) in the Wounds of the Studied Groups on Day 15 According to the ANOVA and LSD Tests. *$P<0.05$, ** $P<0.01$, *** $P<0.001$

of PBM plus physical practice caused a larger decrease in blood insulin levels in comparison with practice plus placebo PBM.[31] Francisco et al reported that PBM with light-emitting diodes plus mild exercise substantially decreased blood glucose values in patients with DM2.[32] Silva et al. reported that one month of daily PBM resulted in reduced blood glucose in obese mice.[33] The results of the above-mentioned studies support our findings that blood glucose levels decreased following PBM in DM1 rats; however, the mechanism by which PBM influences blood glucose metabolism is not completely understood. To the best of our knowledge, the positive effects of PBM on blood glucose levels during the healing course of diabetic skin wounds have not been reported; therefore, the current research is the first study in which the treatment of diabetic wounds with PBM for 15 days caused significant decreases in blood glucose levels. PBM appears to enhance mitochondrial metabolism by rising the activity of CCO oxidase, and this would lead to increased glucose consumption.

Accordingly, a previous study found that PBM increased the activity of citrate synthase and the Krebs cycle. The increased consumption of glucose for ATP creation may explain its reduction in the blood.[34] PBM may also decrease blood glucose levels by inhibiting the c-Jun N-terminal kinase pathway and stimulating the phosphorylation of protein kinase B.[33] However, additional studies in this field are needed.

Approximately 60% of DFUs are complicated by microbial infections. Infections are the most frequent cause of morbidity in DM, resulting in hospitalization and non-traumatic foot amputation.[35] The most common microbes are *Staphylococcus* species. Osteomyelitis is a severe complication of DFUs and increases the need for surgical intervention.[3]. Accordingly, we found that the treatment groups showed a substantial diminution in *S. aureus* CFUs, compared to the control G.

A decreased lack of effectiveness of current DFU treatments creates a need for additional investigations.[37] The healing of DFUs is one of the most important medical problems, making it vital to find effective treatments that are economically feasible.[38] Although research shows that drugs for DM may preclude wounds from becoming arrested in the inflammatory step of healing and that they may promote wound restoration in some cases, to date, Questions remain as to which types of intervention, technology, and dressing are suitable to promote healing, and whether all therapies are necessary and cost-effective as adjunctive therapies.[39]

Recently, there has been significant concern about the clinical use of curcumin for healing wounds, resulting in numerous human studies and publications.[40] Nevertheless, translating these results to clinical settings has been difficult because of the low solubility, instability, and poor assimilation of curcumin.[41] Our previous studies showed that the application of the natural form of curcumin by itself did not increase wound strength and the skin injury restoration process in diabetic rats.[42,43] However, in the current study, the sole administration of CUR did significantly increase wound strength in the DM1 rat model. Advancing the bioavailability of phytochemicals, such as curcumin, researchers have examined the ability of NPs to act as drug transporters, with controlled cargo release and improved pharmacokinetics, bioavailability, and biological efficiency.[44] The instability and weak assimilation of curcumin can be improved by loading curcumin into NPs.[44] We previously assessed the biodistribution of CUR in wounds and healthy skin. A small skin or wound tissue sample was allowed to digest, and the curcumin content was assayed.[23] The wounded skin samples that were exposed to CUR for 72 hours had the highest concentration of curcumin compared to the other samples.

These NPs have small diameters and a large surface area to volume ratio, which raises the chances for biological contact and infiltration into the wound bed. NPs are ideal transporters of bioactive molecules such as curcumin, promoting cell proliferation, vascularization, activation of signaling pathways, and protein synthesis which is essential for productive wound restoration.[45] We showed that when entire treatment groups, compared to the control G, significantly improved wound closure fraction, completely closed wounds, and wound strength, the combination treatment with PBM + CUR was significantly superior to only CUR used alone. The superior results of PBM + CUR could be attributed to the additive effects of PBM and CUR. PBM decreases inflammatory cells, increases fibroblast propagation and angiogenesis, stimulates the formation of granulation tissue, and increases collagen creation.[46] Curcumin promotes cell proliferation, vascularization, and cell signaling,[45] in addition to its anti-inflammatory, antioxidant,[47] and wound restoration properties.[48] Curcumin can restore a lost extracellular matrix by encouraging fibroblast migration and collagen synthesis, which can provide a stable foundation for the formation of granulation tissue.[49]

Our outcomes are in accordance with some previous reports. In a study, Lau et al. explored the impact of gold NPs (GoNPs) plus PBM (diode, 808 nm, 100 mW, 50 s, 5 J/cm^2, 9 sessions) on skin injury repair in rats. Their research contained a control G and two experimental groups that received GoNPs with and without PBM, respectively. While there was no PBM GROUP alone in their study, they determined that the use of GoNPs plus PBM could improve wounds in other groups.[50] Fujimura et al examined the effect of PBM (810 nm, pulse duration: 100 ms, 5 W) plus indocyanine green-loaded nanospheres coated with chitosan (ICG-Nano/c) on keratinocytes as well as an in vivo wound model. They found that, in

addition to its bactericidal effects, PBM alone and PBM plus ICG-Nano/c lessened the inflammatory response and promoted the repair of the injured skin.[51] Kumar et al evaluated the combined effects of silver NPs prepared by green synthesis (G-AgNPs) and PBM (830 nm, 5 J/cm^2) on cultures of non-diabetic injured and diabetic injured fibroblasts. They concluded that combined therapy with G-AgNPs and PBM was beneficial for migration and scratch wound restoration in vitro.[52]

Conclusion

All treatment groups showed significantly improved wound healing in the DM1 rat model. However, the PBM + CUR group was superior to the other treatment groups and the control group in terms of wound strength and stereological parameters. Compared to the control group, the PBM + CUR and PBM groups showed a significant decrease in blood glucose levels.

We propose that a combination of PBM + CUR could be tested in diabetic patients with non-healing wounds to reduce infection and promote healing. We hope our results will open new avenues for the successful treatment of DFUs in patients. Additional studies are required to understand the cellular and molecular mechanisms of PBM, CUR, and their combination in the healing process of cutaneous wounds in diabetic animals.

Authors' Contribution

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Competing Interests

Michael R Hamblin declares the following potential conflicts of interest. Scientific Advisory Boards: Transdermal Cap Inc, Cleveland, OH; Hologenix Inc. Santa Monica, CA; Vielight, Toronto, Canada; JOOVV Inc, Minneapolis-St. Paul MN; Consulting; USHIO Corp, Japan; Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany. The other authors declare that they have no conflicts of interest.

Ethical Approval

The Ethics Committee of Shahid Beheshti University of Medical Sciences approved this study (IR.SBMU.MSP/REC.1395.9124).

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Supplementary Files

Supplementary file 1. Results of statistical analysis

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