



# The Combined Effect of Photobiomodulation and Curcumin on Acute Skin Wound Healing in Rats

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## Abstract

**Introduction:** Abnormal wound repair is a cause for considerable expense, as well as patient morbidity and mortality. Here, we investigated the combined impact of photobiomodulation (PBM) and curcumin on a rat experimental model of an acute skin wound.

**Methods:** A round full-thickness wound was created on the back of each rat. We divided the rats into the following four groups. Group one was the control group. Group two received pulse wave (PW) PBM at a dose of 890 nm, 80 Hz, and 0.2 J/cm<sup>2</sup>. Group 3 received 40 mg/kg curcumin by gastric gavage and group 4 were treated with PWPBM + curcumin. We measured the wound area on days 4, 7, and 15, and performed microbiological and tensiometric examinations.

**Results:** There was markedly improved wound contraction in the curcumin (7.5 ± 0.57; *P*=0.000), PBM (8.5 ± 1.2; *P*=0.000), and PBM + curcumin (14.5 ± 4.3; *P*=0.002) groups relative to the control group (25 ± 6). PBM (100 ± 7.3; *P*=0.005), and PBM + curcumin (98 ± 6; *P*=0.005) groups meaningfully improved tensile strength relative to the control group (61 ± 8.2). On day 15, the PBM (10 ± 5; *P*=0.000), curcumin (14 ± 4.5, *P*=0.000), and PBM + curcumin (27.3 ± 8.3; *P*=0.000) groups meaningfully decreased microbial flora relative to the control group (95 ± 6).

**Conclusion:** We concluded that the PBM and PBM + curcumin groups meaningfully accelerated wound healing of the acute skin wound in the rats. The results of the PBM group were statistically more effective than the curcumin alone and PBM + curcumin-treated groups.

**Keywords:** Wound healing, Curcumin, Therapies, Photobiomodulation, Low-level laser therapy, Wound closure techniques

## Introduction

In 2012, there were approximately 312.9 million surgeries worldwide.<sup>1</sup> Despite recent progress that has emphasized the importance of adjunct therapies in optimizing the repairing potential of skin, abnormal skin repair continues to cause considerable expense, and patient morbidity and mortality.<sup>2</sup>

Curcumin (diferuloylmethane) is an element of the *Curcuma longa* plant<sup>3</sup> and it is considered to be one of the best medicinal herbs because of its anti-microbial, anti-inflammatory, and antioxidant activities.<sup>4</sup> The properties of curcumin have been investigated in different culture systems and animals.<sup>4</sup> The World Health Organization (WHO) confirmed the everyday ingestion of curcumin as a food preservative.<sup>5</sup> Curcumin has also been reported to have remarkable wound healing characteristics. It acts on various stages of the dermal healing course to hasten healing. Curcumin stimulates proliferation and

remodeling stages of the skin repair process and wound contraction, which plays a crucial role in the wound healing process.<sup>6</sup>

The latest investigations demonstrated that treatment of rats with oral gavages of curcumin improved lung injury and decreased mortality,<sup>7</sup> enhanced tendon repair,<sup>8</sup> remarkably moderated the development of osteoarthritis (OA),<sup>9</sup> and had an anti-hypertensive impact.<sup>10</sup>

The use of photobiomodulation (PBM) to reduce pain, inflammation, to promote wound, and to prevent tissue damage has been known for about fifty years since the invention of lasers.<sup>11</sup> Many scientists have documented the beneficial influence of PBM regimens on skin wound healing in humans and animals.<sup>12-16</sup>

Wound healing involves synchronized activities and cellular proliferation. Approximately 9 billion dollars are spent annually in the United States to treat skin wounds. Recent researches have been conducted

on the management of skin wounds.<sup>17</sup> Despite new developments in materials for wound management, conventional treatments that rely on natural elements such as plant juices provide attractive alternatives. These treatments offer novel capabilities for curing dermal injuries, improving access to healthcare, and controlling some of the barriers associated with modern therapeutic products that include elevated costs and extensive production times.<sup>18</sup> Limitations with current skin wound treatments show the need for secure, effective, and inexpensive formulations that can be used to treat skin injuries. Successful treatment would preferably reduce bacterial infections, control the inflammatory reaction, and concurrently increase wound strength.<sup>17</sup>

Combined treatments with different approaches and medicines have been shown to enhance the cure rates of various diseases. Therefore, these combined treatments are a focus of research among scientists today.<sup>19-22</sup> There are few studies that assessed the impact of curcumin on healing an acute skin wound. López-Jornet et al evaluated the effect of topical curcumin on healing a CO<sub>2</sub> laser-induced cutaneous injury in mice. These researchers determined that administration of curcumin to the CO<sub>2</sub> laser-made cutaneous injury might be beneficial because they observed enhanced re-epithelialization after seven days.<sup>23</sup> In another investigation, Moradi et al tested the joined impact of PBM with curcumin-loaded nanoparticles in a simulated acute skin injury mouse model. Moradi et al determined that the combination of PBM and curcumin nanoparticles reinforced the wound contraction and its tensiometric parameters.<sup>24</sup>

Although wounds in patients are closed with suture materials, an adequate amount of inherent strength should be present to maintain the wound closure. Therefore, the complete tensile strength of a wound and minimizing mechanical stressors are vital factors during the postoperative period.<sup>25,26</sup> Nonetheless, we did not find any information in the literature that pertained to the impact of PBM plus curcumin on skin wound healing in normal animals in terms of wound closure, wound strength, and microbiological evaluating methods. In the current study, for the first time, we assayed the combined administration of PBM with curcumin in a rat model of an acute skin injury using wound closure, wound strength, and microbiological evaluating methods.

This study may support the possibility of PBM plus curcumin administration for wounds in patients, especially as management of abnormal skin repairs in large traumatic wounds, large surgical wounds, diabetic foot ulcers, pressure sores, and ischemic ulcers.

## Materials and Methods

### Animals and Study Design

We inflicted a single skin excision on the back of each of 24 adult male Wistar rats. The rats were subsequently randomized into four groups (n=6 per group). Group

one was the untreated control (placebo) group. Group two received pulse wave (PW) PBM. Group three was treated with curcumin. Group four received PWPBM + curcumin. Each rat was kept alone in individual rat polyethylene cages in a standard animal house and provided with standard food and water ad libitum. On days 4, 7, and 15, we measured the wound region and conducted microbiological and tensiometric assays.

### Photobiomodulation

PBM (MUSTANG 2000 with a LO7 probe; Technica Co., Russia) was administered next to the surgery and continued once per day, 6 days per week for 15 days. PBM was performed on 16 defined zones (Figure 1).<sup>23</sup> The PBM parameters were: 1.08 mW/cm<sup>2</sup>, 75W, 1.08 mW, 1 cm<sup>2</sup>, 80 Hz, 890 nm, 180 ns, and 0.2 J/cm<sup>2</sup>, with 200 seconds duration for each laser shot.

### Curcumin Administration

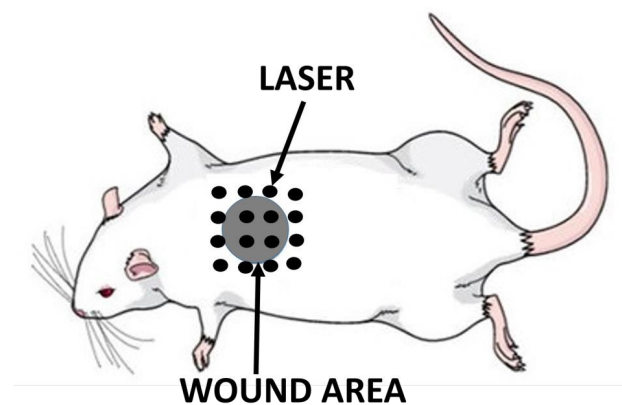
Curcumin (40 mg/kg; Sigma Aldrich, St. Louis, MO, USA) was dissolved in 1 mL sesame oil.<sup>27</sup> At first, a dose-response evaluation with 40, 50, and 60 mg/kg of curcumin was performed. A significant escalation in tensiometrical parameters of the wound healing process by 40 mg/kg curcumin was observed. So in the main study, 40 mg/kg was selected.

### Clinical Observations

The rats' wounds and their weights were inspected and recorded during the experiment.

### Measurements of the Wound Area

In order to compare wound closure among the study groups, we measured the wound area on days 4, 7, and 15. An image was taken of each wound area with an electronic camera, and the surface area (mm<sup>2</sup>) was determined using Adobe Photoshop CS6 extended software. Photography could be applied to estimate the wound area. The boundaries of the wound were defined



**Figure 1.** Schematic Illustration of the Wound Area and Laser-Shooting Targets.

and marked from a digital photograph by using a mouse and a laptop. A ruler was photographed near the wound. The pictures obtained offered a stable record of both the wound size and its appearance.<sup>28</sup>

### Microbiological Examination

We obtained microbial specimens from each wound on days 7 and 15 for the detection of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The samples were primarily cultured and incubated on Mueller Hinton Agar culture. Positive *Staphylococcus* was cultured in Mannitol salt agar for differentiating between *S. aureus* and *S. epidermidis*. We counted the numbers of microbes per sample as colony-forming units (CFUs).<sup>27</sup>

### Tensiometric Examination

The test was clarified precisely in our former probes.<sup>10,13,14</sup> The rats were euthanized and we obtained a standard sample (5 mm × 50 mm band) from each wound and the nearby tissue. The samples were placed in a tensile strength measuring instrument (Santam, Eng. Design Co., Ltd., Iran) that had a deformation rate of 10 mm/min. We reported each sample's bending stiffness (MPa), maximum force (N), stress high load (N/mm<sup>2</sup>), and energy absorption (J). Bending stiffness was calculated by dividing the maximum force by the displacement of the rupture. The maximum force was measured directly from the load-deformation curve and represented the maximum tensile force applied to rupture the specimen. The stress high load was calculated as the maximum force divided by the cross-sectional area of the specimen. Energy absorption was the area under the load-deformation curve. The stress high load was calculated according to the following equation<sup>27</sup>:

Stress high load value = maximum force value (N)/cross-sectional area of the specimen (cm<sup>2</sup>)

### Statistical Analysis

All data are expressed as mean ± standard deviation (SD). Both one-way and two-way analysis of variances (ANOVA) and the least significant difference (LSD) tests were used for data evaluation. A *P* value of <0.05 was considered to be statistically significant.

### Results

All rats successfully tolerated the surgery and the treatment regimens.

#### Wound Closure (mm<sup>2</sup>), One-Way Analysis of Variance, Day 4

All *P* values were related to the LSD test. The curcumin (197 ± 14 mm<sup>2</sup>), PBM (243 ± 20 mm<sup>2</sup>), and PBM + curcumin (231 ± 33 mm<sup>2</sup>) groups showed a meaningful reinforced wound closure relative to the control group (329 ± 6.7 mm<sup>2</sup>) (*P*=0.000). We observed that the curcumin group had meaningfully reinforced wound

closure compared with the PBM (*P*=0.009) and PBM + curcumin groups (*P*=0.040) (Figure 2).

#### Wound Closure, Day 7

The curcumin (69 ± 8.7 mm<sup>2</sup>), PBM (85 ± 12 mm<sup>2</sup>), and PBM + curcumin (88 ± 7.9 mm<sup>2</sup>) groups meaningfully reinforced wound closure relative to the control group (139.8 ± 20.2 mm<sup>2</sup>), (*P*=0.000). We observed that the curcumin-treated group had meaningfully reinforced wound closure relative to the PBM + curcumin group (LSD test, *P*=0.033) (Figure 2).

#### Wound Closure, Day 15

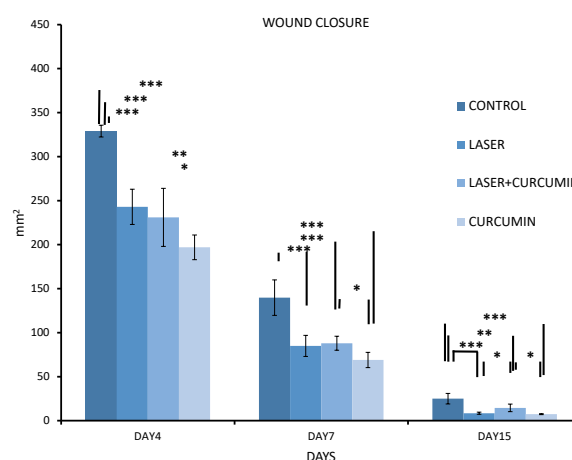
The curcumin (7.5 ± 0.57 mm<sup>2</sup>, *P*=0.000), PBM (8.5 ± 1.2 mm<sup>2</sup>, *P*=0.000), and PBM + curcumin (14.5 ± 4.3 mm<sup>2</sup>, *P*=0.002) groups had meaningfully enhanced wound closure relative to the control group (25 ± 6 mm<sup>2</sup>). According to the LSD test results, the curcumin group (*P*=0.023) and PBM treated groups (*P*=0.045) meaningfully reinforced wound closure relative to the PBM + curcumin group (Figure 2).

#### Two-Way Analysis of Variance

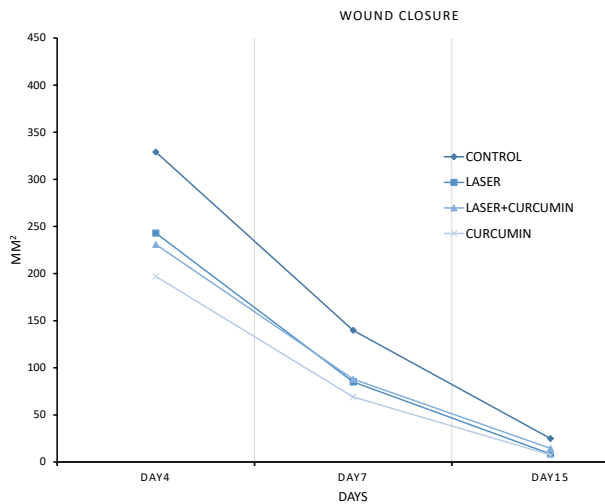
There were statistically significant differences between different intervals and groups. In addition, the interaction between different intervals and the group factors showed that the wound area was much smaller on day 15 relative to the other days. Moreover, the PBM and curcumin groups caused a significant reduction in the wound area on day 15 (Figure 3).

#### Microbiological Analysis, One-Way Analysis of Variance

On day 7, the PBM (10.6 ± 3.5), curcumin (10.3 ± 1.5), and PBM + curcumin (26 ± 4.5) groups had meaningful reductions in CFU relative to the control group (73 ± 3.6)



**Figure 2.** A Comparison of The Mean ± SD for Wound Closures in the Study Groups on Days 4, 7, and 15 by One-Way Analysis of Variance (ANOVA) and the Least Significant Difference (LSD) Test. \* *P*<0.05; \*\* *P*<0.01; \*\*\**P*<0.001.



**Figure 3.** Two-Way Analysis of Variance (ANOVA) Shows a Significant Difference Between Different Time Points and Group Factors. We Found that Two-Way ANOVA Showed Significant Differences Regarding Number of Groups, Number of Time Points, and Interaction Effect as Follows:  $F = 80$ ,  $Sig = 0.000$  For the Number of Groups (4);  $F = 1162$ ,  $Sig = 0.000$  for the Number of Time Points (3); and  $F = 13.7$ ,  $Sig = 0.000$  for the Interaction Effect. Consequently, there is a Meaningful Difference Between Different Time Points and Groups. The Interaction Between Different Time Points and Group Factors Shows that the Wound Area is Much Smaller on Day 15 Compared with the Other Days. The Photobiomodulation (PBM) and Curcumin Groups Caused a Significant Decrease in the Wound Area on this Day.

(all  $P=0.000$ ). The PBM and curcumin groups showed a significant reduction compared to the PBM + curcumin groups (both  $P=0.001$ ). On day 15, the PBM ( $10 \pm 5$ ), curcumin ( $14 \pm 4.5$ ), and PBM + curcumin ( $27.3 \pm 8.3$ ) groups had meaningful reductions in CFU relative to the control group ( $95 \pm 6$ ), (LSD test, all  $P=0.000$ ) (Figure 3). The PBM ( $P=0.009$ ) and curcumin ( $P=0.030$ ) groups showed a significant decrease in CFU compared with the PBM + curcumin groups.

**Two-Way Analysis of Variance**

Microbiological analysis indicated that the treatment with PBM alone and curcumin alone was statistically better than the combined PBM + curcumin treatment (Figure 4).

**Tensiometric Test**

**Bending Stiffness**

Figure 5 shows the tensiometric test results. The PBM + curcumin ( $19.6 \pm 2$ ;  $P=0.002$ ), PBM ( $22.8 \pm 11.2$ ;  $P=0.003$ ), and curcumin ( $19.1 \pm 1.3$ ;  $P=0.006$ ) groups meaningfully increased bending stiffness compared with the control group ( $9.8 \pm 1.4$ ) (Figure 5A).

**Maximum Force**

The PBM ( $10 \pm 0.7$ ) and PBM + curcumin ( $9.8 \pm 0.6$ ) groups meaningfully increased maximum force relative to

the control group ( $6.1 \pm 0.8$ ) (both,  $P=0.005$ ) (Figure 5B).

**Stress High Load**

The PBM ( $100 \pm 7.3$ ) and PBM + curcumin ( $98 \pm 6$ ) groups meaningfully increased the stress high load relative to the control group ( $61 \pm 8.2$ ) (both,  $P=0.005$ ) (Figure 5C).

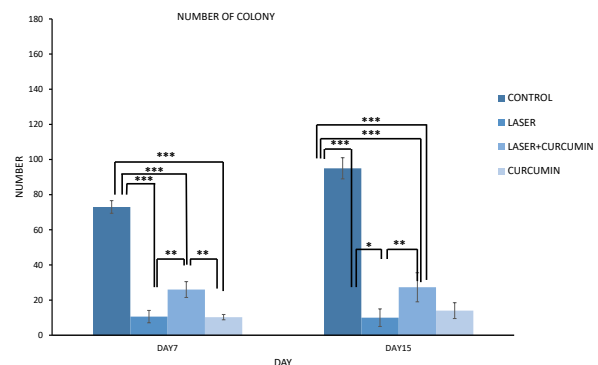
**Energy Absorption**

The PBM + curcumin ( $32 \pm 3.1$ ;  $P=0.003$ ) and PBM ( $31 \pm 2.4$ ;  $P=0.004$ ) groups meaningfully raised energy absorption relative to the control group ( $16 \pm 3$ ).

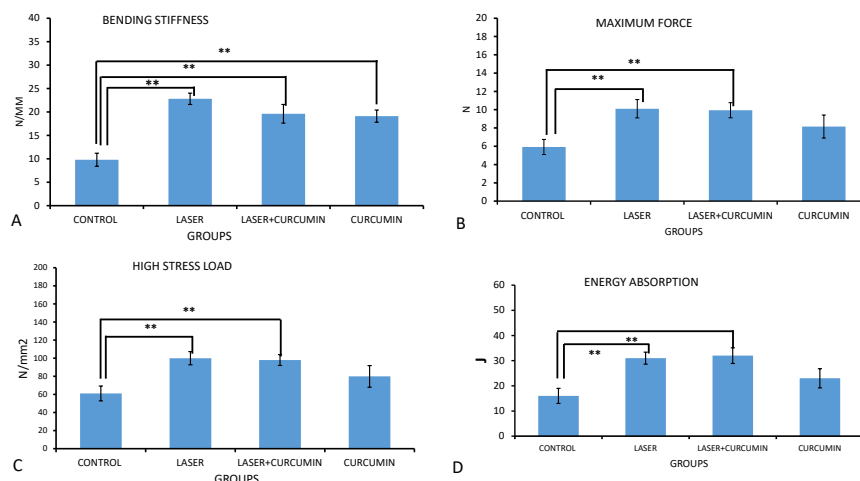
**Discussion**

Wound closure and infection control are the principal objectives of wound management. There are bactericidal agents in the market that customarily reach contamination control. In contrast, wound closure remains challenging. Cell motility and migration have important roles in generating contractile force of patent wound margins and contribute to wound closure.<sup>29</sup> Modulations of these cellular performances have been studied in the framework of wound closure; however, a beneficial tactic to obtain a wound closure has yet to be discovered.<sup>29</sup> The establishment of successful interventions in wound management is an area of interest for investigators.<sup>30</sup> Therefore, medically successful and cost-effective management of wounds is of utmost importance.<sup>31</sup>

Previous data of pretreatment with curcumin in excisional skin wound healing suggests that curcumin plays a potential role in wound healing.<sup>32-34</sup> Jagetia and Rajanikant studied the effect of curcumin on radiation-impaired healing of wounds in mice.<sup>32-34</sup> In their studies,



**Figure 4.** A Comparison Of Mean  $\pm$  SD Colony-Forming Units (CFUs) of The Study Groups on Days 4, 7, and 15 Using One-Way Analysis of Variance (ANOVA) and the Least Significant Difference (LSD) Tests. \*  $P<0.05$ ; \*\*  $P<0.01$ ; \*\*\*  $P<0.001$ . Two-Way ANOVA Results Indicated That  $F = 286$  and  $Sig = 0.000$  for the Number of Groups (4);  $F = 10.5$  and  $Sig = 0.005$  for the Number of Time Points (2); and  $F = 6.6$  and  $Sig = 0.004$  for the Interaction Effect. We Observed Meaningful Differences in CFU Among the Control and Other Groups, and also Among the Photobiomodulation (PBM) and Curcumin Groups and the PBM + Curcumin Group.



**Figure 5.** A Comparison of Mean  $\pm$  SD for Bending Stiffness (A), Maximum Force (B), Stress High Load (C), and Energy Absorption (D) of the Study Groups on day 15 According to One-Way Analysis of Variance (ANOVA) and the Least Significant Difference (LSD) tests. \*\*  $P < 0.01$ .

the skin injury repair was tested by wound closure, mean skin repair times, collagen synthesis, and histologic assessment. However, the researchers did not conduct tensiometric analyses in their studies. The tensiometric parameters of wounds are of vital importance since they indicate the probability of reopening of skin injuries.<sup>35</sup> The patient applies pressure over a repaired wound site, which causes the wound to reopen and it should be retreated.<sup>35</sup> Tensiometric (wound strength) parameters are considered to be the gold standard for evaluating the wound healing process. While many studies have reported the positive impact of curcumin on accelerating wound healing,<sup>6</sup> there have been no studies about the effect of curcumin on the physical (tensiometric) properties of repairing wounds in healthy subjects. Consequently, in the current study, we have examined the impact of curcumin on tensiometric properties of repairing wounds. Our statistical analysis showed that although curcumin did not show a positive effect on wound strength, PBM and PBM + curcumin groups meaningfully increased wound strength compared to the control group (both,  $P = 0.005$ , Figure 5C). It seems the administration of curcumin alone could not accelerate the wound healing process in healthy rats. Moreover, curcumin administration could not improve the beneficial influence of PBM on repairing tissue. Interestingly, in another study, Moradi et al concluded that the topical administration of curcumin nanoparticles, PBM, and PBM+ curcumin nanoparticles increase wound strength.<sup>24</sup> We hypothesized that adding nanoparticles to curcumin increases its absorption in a wound bed compared to the absorption of curcumin alone in the gastrointestinal tract in the current study. Consequently, the positive impact of the curcumin + nanoparticles alone and curcumin + nanoparticles plus PBM on increasing wound strength of repairing tissue was observed.

Some improvements in the tensiometric properties after PBM<sup>16,36,37</sup> suggested an improved wound healing process.<sup>35</sup> In the current study, curcumin did not meaningfully enhance the wound tensiometric properties. Bayat et al reported that PBM (780 nm, 2336 Hz, 2 J/cm<sup>2</sup>) meaningfully increased the fibroblasts, blood vessel sections, and high stress load of a wound bed.<sup>16</sup> Vasilenko et al reported that several power densities of PBM (635 nm, 670 nm, 5 J/cm<sup>2</sup>) improved wound strength.<sup>31</sup> Bisht et al observed that wound strength, early reepithelization, fibroblast number, and neovascularization improved in the laser-treated wounds.<sup>32</sup> Our findings supported the results of these studies.<sup>16,36,37</sup>

In the current study, the reduction in tensiometric properties of the excisional wounds in the curcumin-treated rats was likely attributed to the low doses of curcumin. Recently, Jiang et al examined the influence of curcumin on tendon repair in rats. The test group received 100 mg/kg body weight of curcumin via oral gavage. The researchers found that curcumin remarkably enhanced the repairing characteristics as shown by widespread deposition of well-organized collagen fibrils, reduced malondialdehyde levels, and improved tensiometric properties of the repaired tendon.<sup>8</sup> On the other hand, our pilot study showed a significant escalation in tensiometric properties of an incisional skin wound model in normal rats treated with 40 mg/kg curcumin when evaluated 15 days after surgery.

The results of experiments on the effects of PBM on microbial flora in an *in vitro*<sup>38,39</sup> and an *in vivo* model<sup>40</sup> are inconsistent. PBM increased the wound closure of burns in animals. There was no significant difference in *S. epidermidis*, *S. aureus*, and *P. aeruginosa* growth between the study groups.<sup>40</sup> Some studies have shown the beneficial impact of PBM on the inhibition of bacterial growth *in vitro*<sup>41</sup> and *in vivo*. PBM with dissimilar wavelengths and

energy densities enhanced the wound healing course in infested and non-infested injuries.<sup>15,16,42-45</sup> Ezzati et al examined the effect of PBM (890 nm, 3000 Hz, and 2.3 and 11.7 J/cm<sup>2</sup>) during two probes in rats that had second- and third-degree burns.<sup>15,44</sup> In the first study, they determined that the administration of 11.7 J/cm<sup>2</sup> PBM meaningfully decreased the incidence of *S. aureus* and meaningfully increased the wound-closure rate compared with the placebo in the second-degree burns.<sup>15</sup> In the second study, the researchers reported that the incidence of *S. epidermidis* meaningfully decreased and meaningfully increased the wound-closure rate after the administration of 11.7 J/cm<sup>2</sup> PBM.<sup>44</sup>

Based on our results and data reported from other studies,<sup>15,44</sup> the administration of PBM on a wound is crucial and a PBM protocol with proper energy density is necessary to achieve maximum bactericidal effects along with enhanced wound healing.

In many illness courses, the course of happenings contributing to wound healing could be negatively affected, resulting in chronic, non-healing wounds that subject the patient and community to significant discomfort, distress, and bills. The healing of a skin injury needs numerous elements to incorporate together in concert, and wound dressings and treatments have developed significantly to overcome probable blocks to wound healing, ranging from infection to hypoxia. Even ideally, repairing tissue does not ever grasp its pre-injured power and many abnormal healing states could result in chronic wounds.<sup>46</sup> Wound care is a major healthcare expenditure. Treatment of burns, surgical and trauma wounds, and skin wounds is a major medical challenge with current therapies largely focused on supportive care measures.<sup>47</sup> Consequently, there is a trend among researchers to apply several beneficial agents for accelerating wound healing at the same time.<sup>48-50</sup>

We concluded that the PBM and PBM + curcumin treated groups meaningfully accelerated wound healing of an acute skin wound in rats. The results of the PBM-treated group were statistically more effective than the curcumin alone and PBM + curcumin-treated groups.

The importance of wound healing and the benefits of PBM and curcumin are documented. Because of the limited studies that have evaluated the impact of PBM and curcumin on wound healing, we suggest that additional studies assess the impact of different curcumin administration models such as curcumin nanoparticles to determine the best effect on non-healing wounds such as diabetic wounds in animal models and patients.

### Ethical Considerations

The Institutional Review Board of Shahid Beheshti University of Medical Sciences, Tehran, Iran approved this study (protocol: 9124).

### Conflict of Interests

The authors declare that there are no conflicts of interest.

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