Original Article

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Curcumin Enhances the Reduction of Keloid Fibroblast Viability Induced by Blue Light

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Abstract:

Objective: This study aims to determine the effects of blue light or curcumin alone and its combination on keloid fibroblast viability.

Material and Methods: Human keloid fibroblasts were divided into different groups: untreated controls, blue light irradiation at 30 J/cm^2, curcumin treatment (1.25, 2.5, 5, 10, and 20 μg/mL), and curcumin combined with blue light.

Results: Cell viability was assessed using an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and curcumin's half-maximal inhibitory concentration (IC50) was calculated through regression analysis. Blue light at 30 J/cm^2 significantly reduced keloid fibroblast viability to 61.99%, while curcumin at 20 μg/mL decreased viability to 72.01%. When combined with blue light, curcumin exhibited a dose-dependent effect: 5 μg/mL of curcumin reduced viability to 36.51%. In contrast, 10 and 20 μg/mL decreased viability to 15.94% and 15.85%, respectively—significantly greater reductions than curcumin alone. Blue light enhanced curcumin's effects, lowering its IC50 from 30.95 μg/mL to 3.86 μg/mL.

Conclusion: The combination of blue light and curcumin demonstrated synergistic effects, achieving greater reductions in keloid fibroblast viability compared to either treatment alone.

Keywords: blue light, curcumin, fibroblasts, keloid, photosensitizing agent

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Introduction

Keloid is a fibroproliferative disorder characterized by excessive wound healing, resulting in the overgrowth of connective tissue that protrudes above the skin surface[1]. Keloids are associated with symptoms such as pruritus, pain, mechanical dysfunction, and aesthetic concerns, which can significantly impair the quality of life[2]. High recurrence rates and variable efficacy hinder existing keloid treatment modalities, including intralesional corticosteroid injections, surgical excision, electrosurgery, cryosurgery, and laser therapy. Consequently, there is a pressing need for safer and more effective therapeutic alternatives, such as blue light phototherapy[3].

Blue light, a form of visible light with a 400–500 nm wavelength, is non-toxic to the skin and exhibits properties akin to ultraviolet (UV) light but with fewer side effects[4]. It inhibits transforming growth factor-β1 (TGF-β1) and suppresses fibroblast-to-myofibroblast differentiation[5]. Additionally, blue light induces reactive oxygen species (ROS) production, promotes apoptosis[6], and facilitates collagen degradation by upregulating matrix metalloproteinase-1 (MMP-1) through the c-Jun N-terminal kinase (JNK) and epidermal growth factor receptor (EGFR) pathway[7]. Furthermore, blue light enhances the expression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), inducing oxidative stress and cell death[8].

Due to its limited skin penetration, photosensitizers such as curcumin can enhance blue light efficacy, which has an absorption peak of 450–495 nm[9]. Curcumin, a natural compound derived from *Curcuma longa* (turmeric), exhibits anti-inflammatory, antioxidant, and antiproliferative properties[10]. This study aims to investigate the synergistic effect of curcumin and blue light in reducing keloid fibroblast viability, hypothesizing that the combination therapy is more effective than blue light monotherapy.

Material and Methods

This study is an in vitro experimental investigation utilizing human keloid fibroblast samples. The research was conducted at the Laboratory of the Department of Dermatology and Venereology and the Department of Pharmacology and Therapy, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta. The experimental design comprised: (1) fibroblast cultures without treatment (negative control), (2) fibroblasts exposed to blue light 30 J/cm^2 (positive control), (3) fibroblasts treated with curcumin at five different concentrations i.e. 20 µg/mL, 10 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL, and (4) fibroblasts treated with curcumin at the same five concentrations and exposed to blue light.

Blue light with a 415–455 nm wavelength was emitted by an LED lamp (PDT Biolight Standing®) at a dosage of 30 J/cm^2. This dosage was chosen based on previous literature and our pre-elimminary study that showed in comparison to the nonirradiated group, blue light irradiation at 30 J/cm^2 reduced the formation of ROS in human dermal fibroblasts, increased the expression of matrix metalloproteinase (MMP)-1 and 3, and decreased the expression of collagen types 1 and 3[11]. Curcumin, extracted from *Curcuma longa* (turmeric) and sourced from Sigma Aldrich, was diluted in Dulbecco's Modified Eagle Medium (DMEM) to achieve final concentrations of 20 µg/mL, 10 µg/mL, 5 µg/mL, 2.5 µg/mL, and 1.25 µg/mL. These concentrations were based on previous studies demonstrating the IC50 of curcumin on the viability of various cells, which was 5.58 to 13.9 µg/mL[12,13].

Fibroblasts cell culture

Human fibroblasts were cultured in DMEM media and subcultured to passage 4. Fibroblast cultures were derived from human prepuce skin tissue collected during circumcision procedures. The criteria for inclusion involved dermal skin obtained from the prepuce of healthy individuals under the age of 12. The criteria for exclusion were the presence of infection in the prepuce skin.

The tissue collected was subsequently transferred from the transport medium (DMEM+Penicillin-Streptomycin

2%+Fungizone 2%) and placed in a 6-mm petri dish with 6 mL of fetal bovine serum (FBS). It was soaked in 10% povidone-iodine solution for 3 minutes, then thoroughly washed with sterile phosphate-buffered saline (PBS) until the povidone-iodine color was completely removed. The epidermal and dermal layers were meticulously separated with sterile tweezers. The dermis tissue was subsequently sectioned into pieces measuring 0.5x0.5 cm. The dermis tissue, which has been sectioned, was carefully placed into small flasks with Pasteur's tweezers. One milliliter of complete DMEM medium was added to each flask before incubation at 37 °C with 5% CO_2 for 24 hours. The DMEM medium was replaced daily for three days or until the tissue was fully attached. Once attachment was achieved, ensuring the medium completely enveloped the tissue was essential. The fibroblasts reached the appropriate confluence for subculture once they occupied 50% of the flask surface area.

Cell viability assay

The cell viability assay was conducted at the Department of Dermatology and Venereology Laboratory, Universitas Gadjah Mada (UGM) Department of Dermatology and Venereology laboratory. The BIO-Light® Brand LED Skin Rejuvenation tool, calibrated by the National Standardization Agency with a 95% confidence level, was used for blue light exposure. Measurements taken at a 4 cm distance indicated an irradiation value of 54.72 watts/m^2, equivalent to 0.005472 watts/cm^2. Before exposing the test group, viability was assessed in the control group using various doses of blue light.

The viability of fibroblasts was evaluated 24 hours following exposure to blue light. A solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) at a concentration of 100 µg/mL was introduced to fibroblast cultures and incubated at 37 °C for 3 hours. The outcome was the precipitation of formazan, which was then dissolved in dimethyl sulfoxide (DMSO). The absorbance value was recorded using a spectrophotometer set to a wavelength of 542 nm. The potential of fibroblasts can be determined through the analysis of absorbance results. Fibroblast viability was determined by comparing each test group's average optical density (OD) to the control group, with the control OD value set as 100%. The viability of fibroblast cells was assessed at a single time point, specifically at 24 hours.

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 26. The Shapiro-Wilk test (p-value<0.05) was used for normality testing. Group differences were analyzed using one-way ANOVA (p-value<0.05) and LSD post-hoc tests to compare treatment groups. The half-maximal inhibitory concentration (IC50) of curcumin, calculated as the concentration required to inhibit 50% of keloid fibroblast viability, was obtained through probit analysis (95% confidence interval) using SPSS version 26 software (IBM Corp., USA).

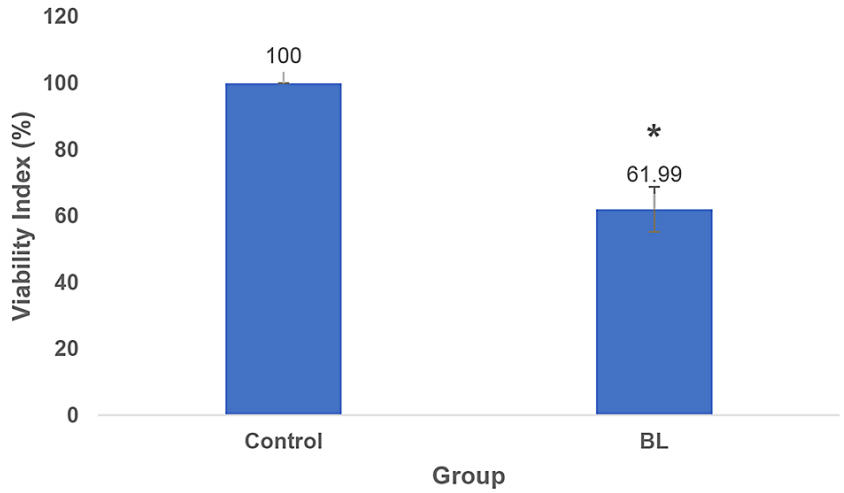
Ethics

This research received ethical clearance from the Biomedical Research Ethics Committee of Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada (FKKMK) UGM (Ref. No. KE/FK/0687/EC/2024), issued on May 15, 2024. Data confidentiality was maintained, and all information was used exclusively for research purposes.

Results

Effect of blue light exposure on keloid fibroblast viability

The control group, comprising untreated fibroblast cultures, was used as the reference, with its viability set at 100%. Fibroblast cultures exposed to blue light (30 J/cm^2) demonstrated a viability of 61.99%±6.79 (Figure 1). This result indicates that blue light exposure at this dose



*=p-value<0.05 compared to the control group.

Figure 1 Keloid viability index without blue light exposure (control) and blue light exposure (BL), Control: Untreated keloid fibroblast cell culture (10,000 cells/well), BL=Keloid fibroblast culture exposed to blue light (30 J/cm^2)

significantly reduced fibroblast cell viability by 38.01% (Figure 1).

Effect of curcumin on keloid fibroblast viability

Keloid fibroblast cultures treated with curcumin at various concentrations exhibited differing viability indices. At lower doses (2.5 µg/mL and 1.25 µg/mL), curcumin slightly increased the fibroblast viability index by 1.83% and 7.47%, respectively. A reduction in viability index was observed at 5 µg/mL (9.1%) and 10 µg/mL (8.43%), though these changes were not statistically significant. However, at 20 µg/mL, curcumin caused a substantial 27.99% reduction in viability (27.99%, p-value<0.001) (Figure 2).

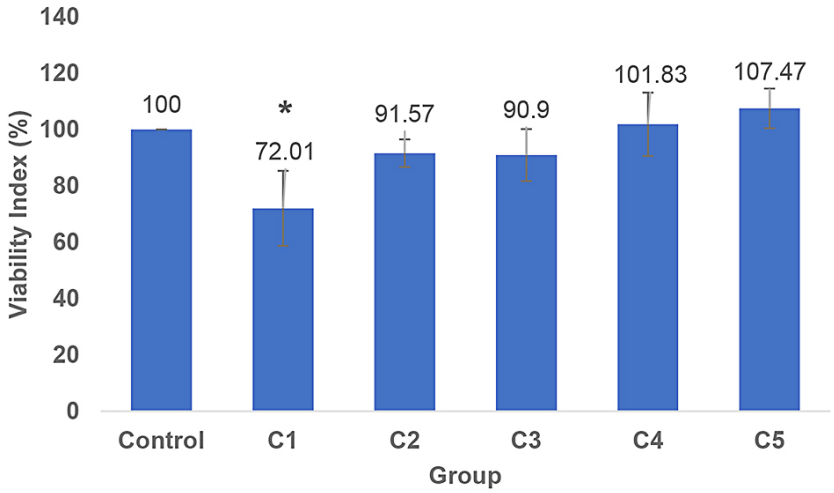
Effect of combined blue light and curcumin on keloid fibroblast viability

The combined effects of blue light (30 J/cm^2) and curcumin on fibroblast viability were evaluated against the negative control (untreated fibroblasts) and positive control (BL group, viability=61.99%±6.79). Fibroblast viability levels in groups treated with blue light and curcumin at various doses are shown in Figure 3.

All treatment groups significantly reduced fibroblast viability compared to the negative control. Compared to the BL group, significant reductions were observed in groups BLC1, BLC2, and BLC3. In BLC4 (2.5 µg/mL), there was a slight decrease (2.74%), which was not statistically significant. In BLC5 (1.25 µg/mL), the viability level exceeded that of the BL group, indicating no further decrease. These findings suggest that higher doses of curcumin when combined with blue light, result in a more significant reduction in fibroblast viability. Conversely, lower curcumin doses did not significantly enhance the reduction in viability (Figure 3).

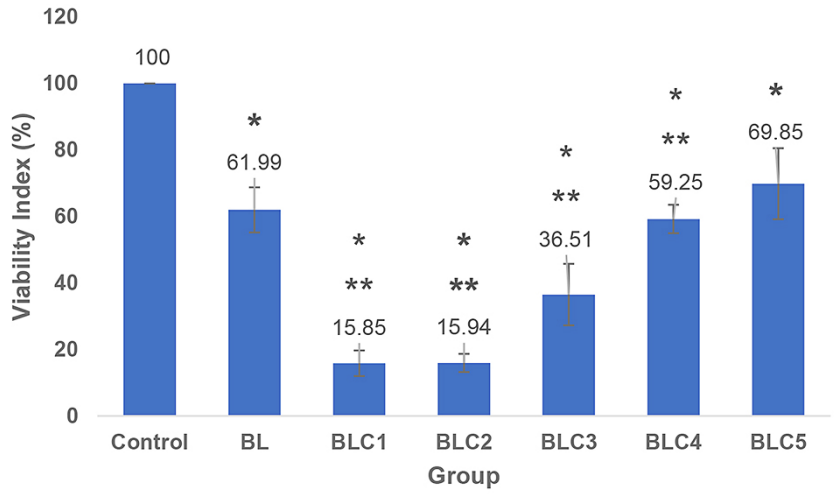
Effect of curcumin with and without blue light on keloid fibroblast viability

The study demonstrated that curcumin at a dose of 20 µg/mL, when used as a single agent, effectively reduced fibroblast viability. However, combining curcumin and blue



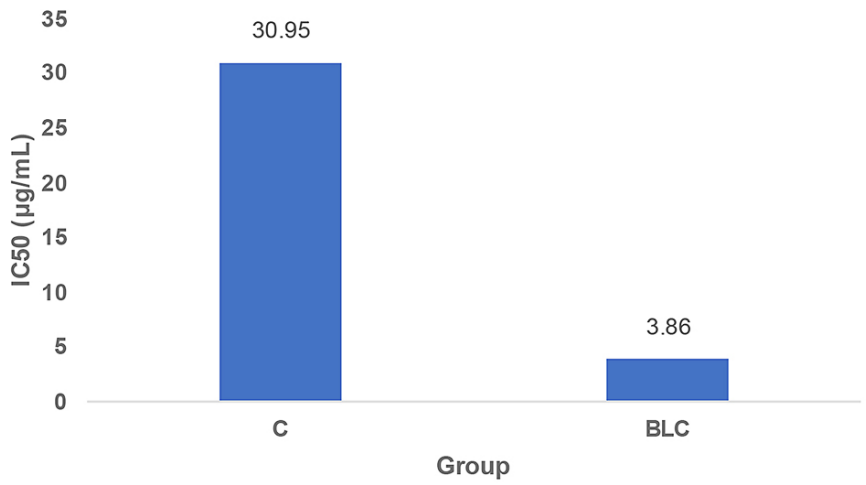
*=p-value<0.05 compared to the control group

Figure 2 Keloid fibroblasts viability index of the control and curcumin-treated group, Control=untreated keloid fibroblast culture (10,000 cells/well), C1=curcumin 20 µg/mL; C2=curcumin 10 µg/mL, C3=curcumin 5 µg/mL, C4=curcumin 2.5 µg/mL, C5=curcumin 1.25 µg/mL



*=p-value<0.05 compared to the control group, **=p-value<0.05 compared to the BL group

Figure 3 Keloid fibroblast viability index of the control group and the fibroblast groups exposed to blue light combined with curcumin at various doses, Control=untreated keloid fibroblast culture (10,000 cells/well); BL=keloid fibroblast culture exposed to blue light (30 J/cm^2), BLC1=blue light+curcumin 20 µg/mL, BLC2=blue light+curcumin 10 µg/mL, BLC3=blue light+curcumin 5 µg/mL, BLC4=blue light+curcumin 2.5 µg/mL, BLC5=blue light+curcumin 1.25 µg/mL



*=p-value<0.05 for groups with similar curcumin doses without blue light exposure

Figure 4 Keloid fibroblast viability index of the control group (with and without blue light exposure), curcumin-treated group, and the keloid fibroblast groups exposed to blue light combined with curcumin at various doses, Control=untreated keloid fibroblast culture (10,000 cells/well), BL=keloid fibroblasts exposed to blue light (30 J/cm^2), C1=curcumin 20 µg/mL, C2=curcumin 10 µg/mL, C3=curcumin 5 µg/mL, C4=curcumin 2.5 µg/mL, C5=curcumin 1.25 µg/mL, BLC1=blue light+curcumin 20 µg/mL, BLC2=Blue light+curcumin 10 µg/mL, BLC3=blue light+curcumin 5 µg/mL, BLC4=blue light+curcumin 2.5 µg/mL, BLC5=blue light+curcumin 1.25 µg/mL

light (30 J/cm^2) resulted in a more substantial reduction in fibroblast viability across all treatment groups (Figure 4).

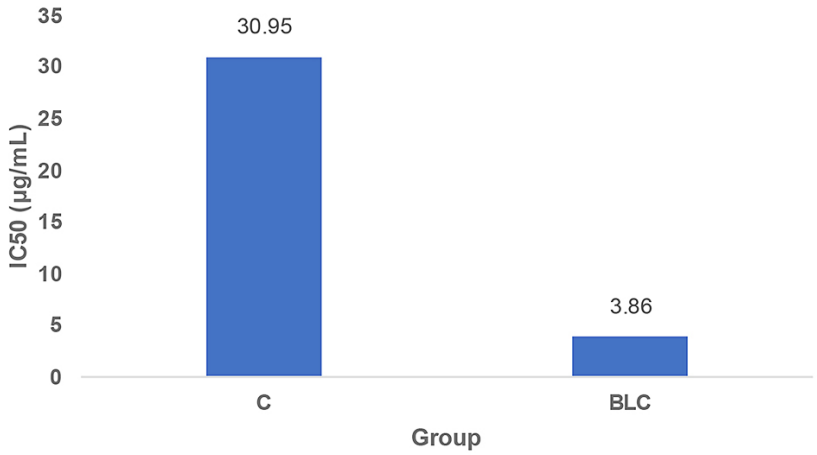
Curcumin at a 5 µg/mL concentration showed notable efficacy as a photosensitizer for blue light, decreasing fibroblast viability. The data further indicate that increasing the dose of curcumin in blue light therapy proportionally decreases fibroblast viability. However, while the observed reductions are promising, the correlation between fibroblast viability reduction and actual clinical improvement in keloid size remains unclear (Figure 4).

Half-maximal inhibitory concentration (IC50) of curcumin with and without blue light

The half-maximal inhibitory concentration (IC50) of curcumin—defined as the concentration required to reduce fibroblast viability by 50%—was determined using probit analysis. In the group treated with curcumin alone (no blue light exposure), the IC50 was calculated to be 30.95 µg/mL. When curcumin was combined with blue light exposure, the IC50 was significantly reduced to 3.86 µg/mL (Figure 5). The IC50 of curcumin combined with blue light was approximately 8 times lower than the IC50 of curcumin as a single agent, indicating a substantial enhancement in efficacy when curcumin is used as a photosensitizer for blue light.

Discussion

The study demonstrates the effectiveness of combining blue light and curcumin in significantly reducing keloid fibroblast viability, exhibiting synergistic effects that independently exceed each treatment's. Blue light (400–500 nm) has been demonstrated to minimize fibroblast viability by processes that include ROS generation, MMP-1 activation, and transforming growth factor-beta 1 (TGF-β1)



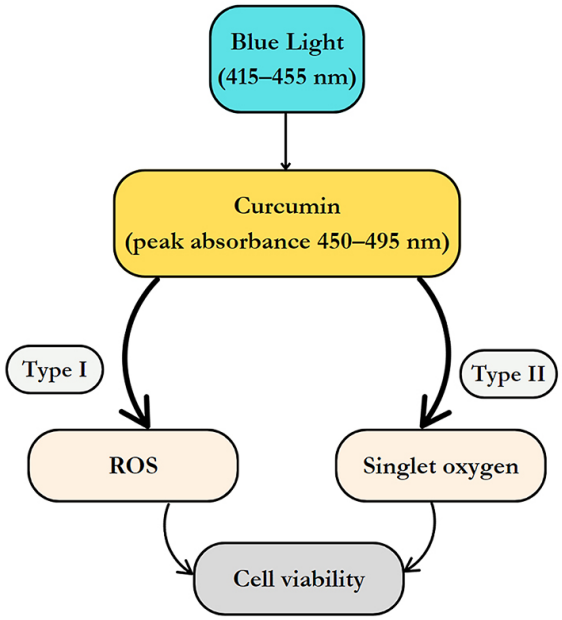
C=curcumin, BLC=blue light+curcumin

Figure 5 Differences in the half-maximal inhibitory concentration (IC50) of curcumin with and without blue light exposure on keloid fibroblast viability

suppression, which are essential pathways involved in fibroblast proliferation and collagen deposition. As shown in Figure 6, blue light could induce type I and type II reactions in curcumin as a natural photosensitizer to affect cell viability[16]. These results correspond with Montero et al. (2023)[14], who reported a substantial decrease in fibroblast viability after exposure to blue light. Similarly, Brüning et al. (2023)[15] demonstrated the dose-dependent effects of blue light, revealing that higher doses, particularly 20 J/cm^2, reduced fibroblast viability by approximately 25%, reinforcing the hypothesis that increased blue light intensity enhances cytotoxicity.

Curcumin, a natural photosensitizer, enhances the therapeutic efficacy of blue light by intensifying its cytotoxic effects. At elevated concentrations, curcumin demonstrates pro-oxidant properties, hence enhancing ROS generation and triggering death in affected fibroblasts. The observed effects align with the findings of Vázquez-Alberdi et al. (2024)[17] who documented the dose-dependent cytotoxicity of curcumin. Lee et al. (2022)[18] also confirmed curcumin's ability to generate ROS-mediated cytotoxicity in both malignant and normal cells, affirming its dual function as an efficient therapeutic agent and photosensitizer. The IC50 of curcumin considerably decreased from 30.95 µg/mL in monotherapy to 3.86 µg/mL in combination therapy, highlighting the synergy between curcumin and blue light.

The results correspond with Scharstuhl et al. (2009)[19], who indicated that elevated doses of curcumin might induce fibroblast death, and Ma et al. (2017)[20], who illustrated curcumin's antifibrotic characteristics. Curcumin regulates TGF-β1 and MMP-9 expression, inhibits fibroblast development into myofibroblasts, and prevents excessive collagen accumulation, which is crucial in treating fibrotic disorders such as keloids. Furthermore, curcumin's absorption spectrum (300–500 nm) overlaps with blue light emission, enhancing its efficacy as photodynamic therapy (PDT) photosensitizer. Strazzi-Sahyon et al. (2022)[21] confirmed this synergy, demonstrating greater fibroblast apoptosis when curcumin (1,000 mg/L) was administered alongside blue light exposure.



ROS=radical oxygen species

Figure 6 Type I and type II reactions in curcumin as natural photosensitizer for blue light that affects cell viability (Adapted and modified from Polat and Kang, 2021)[16]

Curcumin's capacity to augment blue light therapy through photosensitization is supported by Zheng et al. (2022)[22], who documented its effectiveness in reducing inflammation and bacterial growth. Manoil et al. (2014)[23] demonstrated that curcumin significantly reduced the viability of Streptococcus mutans under blue light therapy. In contrast, Woźniak et al. (2021)[24] emphasized its phototoxic effects on normal and malignant skin cells, promoting apoptosis and necrosis in cancerous cells.

This research highlights the efficacy of curcumin combined with blue light as a complementary, non-invasive method for addressing keloid fibroblasts. Curcumin amplifies blue light's cytotoxic effects and offers supplementary antifibrotic advantages, rendering this combination a promising therapeutic strategy for addressing fibroblast-mediated illnesses. The limitation of our study is the study design and protocols. Our study design is in vitro. The protocols, including drug penetration and light diffusion, were heterogeneous, so it may differ in clinical settings. Curcumin as a natural photosensitizer, has shown a potential in increasing the therapeutic effect of blue light therapy against keloid. Further research is needed to optimize dosing, assess potential side effects, and evaluate long-term outcomes to enhance clinical applicability and safety. These initiatives may establish curcumin-blue light therapy as a routine treatment for keloid management and other fibroblast-related conditions.

Conclusion

The combination of blue light and curcumin demonstrated synergistic effects, achieving greater reductions in keloid fibroblast viability compared to either

treatment alone. Blue light exposure markedly enhanced curcumin's potency, reducing its IC50 from 30.95 μg/mL to 3.86 μg/mL.

Acknowledgement

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Conflict of interest

We declare no conflict of interest in our study.

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