# The Effect of Compact Fluorescent Lamp and Curcumin on Wound Healing in Wistar Rats

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**Background:** Nowadays, the rapid recovery of skin lesions and functional return are among the goals of researchers. The skin is the first defensive barrier against microorganisms in the body and its failure causes infection to spread in all systems of the body. By taking into account the contradictory results of previous studies on the impact of phototherapy on wound healing and also the considerable anti-oxidative properties of curcumin, this novel study was carried out with the aim of determining the histopathological impact of compact fluorescent light (CFL) and curcumin on the process of wound healing.

**Methods:** Forty-eight adult male wistar rats were randomly divided into four groups. The control group received 2.0 ml of ethyl oleate, and the curcumin group received only 0.2 ml curcumin daily for 15 days via intraperitoneal injection. The fluorescent group received 0.2 ml of ethyl oleate daily for 15 days via intraperitoneal injection, and were exposed to CFL for 12 hours per day for 15 days. The curcumin plus fluorescent group received 0.2 ml curcumin daily for 15 days via intraperitoneal injection, and were exposed to CFL for 12 hours per day for 15 days. The size of the wound was measured by a scale ruler, and the morphology of the wound site was assessed.

**Results:** The results of this study showed that the best percentage of repair was observed in the fluorescent group on days 6 and 15 (50±5 and 90±2, respectively), while the least repair was seen in the group receiving fluorescent plus curcumin (33±7). In the curcumin group, the wound healing was, not significantly ($P=0.872$) reduced on the sixth day, compared to the control group, whereas compared to the fluorescent and fluorescent plus curcumin groups, the reduction was significant ($P<0.0001$ and $P=0.05$, respectively). On the fifteenth day, however, the wound healing was significantly decreased in the curcumin group compared to the control and fluorescent groups ($P<0.0001$ and $P<0.0001$ respectively), while it was significantly increased compared to the fluorescent plus curcumin group ($P<0.0001$). In the fluorescent plud curcumin group, the wound healing was significantly reduced compared to the other groups on the fifteenth day ($P<0.0001$).

**Conclusion:** Fluorescent alone resulted in wound healing, in contrast to the control and curcumin plus fluorescent groups. Accelerating the repair in this group is likely due to the increase in blood flow and helping the homeostasis to return to its primary state. The absence of wound healing in the curcumin group is probably due to the high dose of curcumin. Moreover, in the fluorescent plus curcumin group, the causes of no wound healing and weight loss were probably disorders in the inflammation process and spread of infection.

**Keywords:** wound, wound healing, compact fluorescent lamps, curcumin

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## INTRODUCTION

The human body is comprised of many organs, the largest of which is the skin, which prevents the entry of many microorganisms and significantly reduces water loss [1]. As part of the integumentary system, its integrity and function are constantly threatened by an array of insults [2].

Proper wound healing and reconstruction is a sophisticated procedure, harmonized between the functions of inflammatory and local cells, components of the extra cellular matrix and other mediators [2]. Wound healing consists of three phases: inflammation, proliferation and remodelling. During the inflammatory phase, platelets aggregate, clots appear and inflammatory cells migrate to the site of insult. In the proliferation phase, keratinocytes, fibroblasts, and endothelial cells proliferate and the new tissue starts growing. Angiogenesis and collagen deposition at the site occur during this phase. The phase may last from two days to three weeks, resulting in a "granulation tissue". During the remodelling phase, excessive cells are removed and collagen is realigned along the tension lines, which might take six months to two years to complete [3,4].

Over the recent years, a great amount of effort has been made to improve wound healing. Some studies have tested various extracts, resins and ointments. Curcumin, the main chemical component of Curcuma longa, commonly known as turmeric, is amongst the most importantly known herbal medications, used for centuries as a potential cure for many diseases. In traditional medicine practiced in Iran and India, turmeric is used to treat respiratory, hepatic and digestive diseases as well as sinusitis. It has further been used to dry up wounds and relieve the wounded of their pain [5,6]. It has recently been proven, both in vitro and in vivo, that curcumin possesses anti-oxidative, anti-inflammatory, anti-amyloidal and anti-arthritic properties [7-9].

Ultra violet (UV) light has also been the subject of various studies in the field of wound healing. Depending on the wavelength, duration of exposure and intensity, UV light may have both positive and negative effects on wound healing [10]. Recent studies have revealed that UV light is capable of restoring homeostasis and possesses anti-inflammatory and anti-oxidant properties, conducing to the process of repair and regeneration [11,12]. On the contrary, a number of studies have provided evidence against the foregoing statements; among the results of these studies, mention can be made of immune system suppression, damage to the DNA, initiation of apoptosis, increased risk of developing skin cancers and tumour formation, oxidative stress injuries and insults to the ophthalmic system [13,14].

By taking into account the conflicting results of previous studies concerning the impact of phototherapy on wound healing and also the considerable anti-oxidative properties of curcumin, the present research was designed with the aim of determining the histopathological impact of compressed fluorescent light and curcumin administration on the process of wound healing.

## MATERIALS AND METHODS

The animal studies were approved by Institutional Animal Care and Use Committee of Urmia University of Medical Sciences.

### Animals

We obtained 48 Wistar rats (gender: male; age: 3–4-months; weight: 200±30 gr) from our Institutional Animal Care Unit. After acclimation, the animals were held in our experimental animal unit under a cycle of 12 hrs of dark and 12 hours of light (humidity: 50-60%; temp: 25 degrees Celsius). A balanced diet with uncontrolled access to food and water were provided. The animals were treated as mandated by the Helsinki Declaration.

### Study Design

Anaesthesia was performed by intraperitoneal injection of ketamine hydrochloride (Alfamine®, Alfasan International BV, Woerden, Holland) and xylazine HCl (Alfazyne®, Alfasan) (100 mg/kg and 0.05 mg/kg, resp.). Following anaesthesia, the back of the animal was shaved and a punch was used to obtain skin biopsies (1/5×1/5 cm) (day 0).

Rats were randomly divided into four evenly distributed groups. The control group (n=12) received 2.0 ml of ethyl oleate daily for 15 days via intraperitoneal injection, without the administration of curcumin and exposure to CFLs. The curcumin group received only 0.2 ml (20 µM) curcumin daily for 15 days via intraperitoneal injection. The fluorescent group (n=12) received 0.2 ml of ethyl

oleate daily for 15 days via intraperitoneal injection and were exposed to CFL for 12 hours per day for 15 days. The curcumin plus fluorescent group (n=12) received 0.2 ml (20 µM) curcumin daily for 15 days via intraperitoneal injection and were exposed to CFL for 12 hours per day for 15 days.

The rats were anesthetized at the end of the experiment. Blood samples were collected and serum was isolated from the blood samples, at which point, the levels of TNFα were measured. The wound size was measured by a scale ruler on a daily basis, the dimensions of the wound were specified, and the morphology of the wound site was assist.

### Histological Evaluation

The sample tissues were stained with Hematoxylin -and-eosin staining. The slides were viewed using light microscopy and under varying magnifications, with the aim of obtaining evidence of re-epithelialization, blister crusting, spongiosis, granulation tissue formation, collagen matrix organization, inflammation, congestion and edema.

### Statistical Analysis

Data was analysed using SPSS software (version 21, Chicago, IL, USA) by non-parametric tests of Kruskal-Wallis and Mann-Whitney with bonferroni correction for multiple comparisons. Continuous data are presented as means ± standard deviations. A $p<0.05$ was considered as statistically significant for Kruskal-Wallis and corrected for Mann-Whitney.

## RESULTS

### Skin repair percentage at days 0, 6 and 15

The most percentage of repair was observed in the fluorescent group on days 6 and 15 (50±5% and 90±2 %, respectively), while the least was observed in the group receiving fluorescent and curcumin (33±7%). The mean percentage of repair in the fluorescent group and the control, and the curcumin and curcumin with fluorescent groups showed a significant increase on day 6 ($P<0.0001$, $P<0.0001$ and $P<0.0001$, respectively). Moreover, comparing the means of repair in fluorescent and curcumin group with control and curcumin groups, a significant increase was observed ($P=0.03$, $P<0.0001$ respectively); but compared to the fluorescent group, on the other hand, a significant decrease was seen. On day 15, comparison of the mean percentage of repair in the fluorescent group

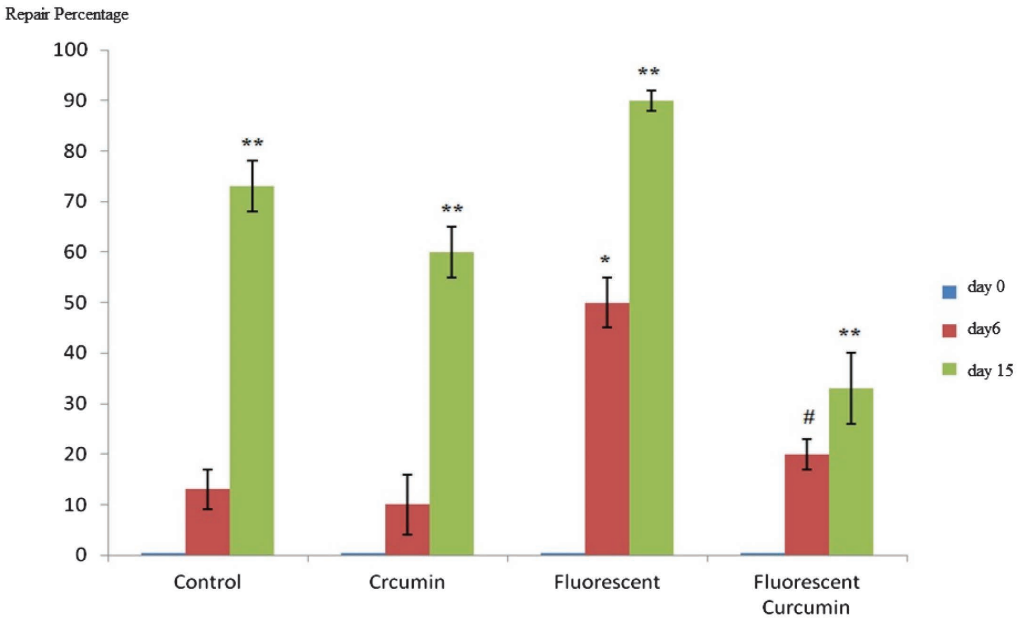


**Figure 1.** Comparison of average skin repair percentage
Comparing average skin repair percentage in mature male Wistar rats on days 0, 6 and 15 between control, curcumin, fluorescent, and curcumin plus fluorescent groups. Data are presented as means and SDs.
*significant compared to control, curcumin, curcumin plus fluorescent groups on day 6.
** Meaningful when compared to each other in control, curcumin, fluorescent, curcumin plus fluorescent groups on day 15.

with the control, curcumin and curcumin with fluorescent groups showed a significant increase (P<0.0001, P<0.0001, P<0.0001 respectively). In addition, a significant decrease was observed in fluorescent and curcumin group when compared to controls and curcumin groups. Healing was significantly better in the controls compared to the curcumin group. The results of analyses on day 15 revealed that in groups receiving curcumin, skin repair was significantly reduced compared to the groups not receiving curcumin (Figure 1).

## Macroscopic evaluation of injury site at day 15

Macroscopic evaluation of injured skin on days 0, 6 and 15 in curcumin, fluorescent plus curcumin with fluorescent groups revealed that on day 15, a significant improvement was observed in fluorescent group compared to other groups. The control group also improved compared to other groups on day 15. As presented in Image 1, a decrease in body mass was observed in the florescent plus curcumin group.

## Morphological finding at injury site on days 6 and 15

Histologic evaluation of the site of injury in the control group on day 6 revealed the absence of hair follicles, sweat and sebaceous glands. However, these were also observed at areas adjacent to the injury site (A). In groups receiving curcumin, hair follicles at injury site and adjacent sub epidermis, sweat glands at adjacent sub epidermis were observed (B). Moreover, tissue assessment of fluorescent group on day 6 revealed the more frequent presence of sebaceous and sweat glands compared to the control group (C). The observation

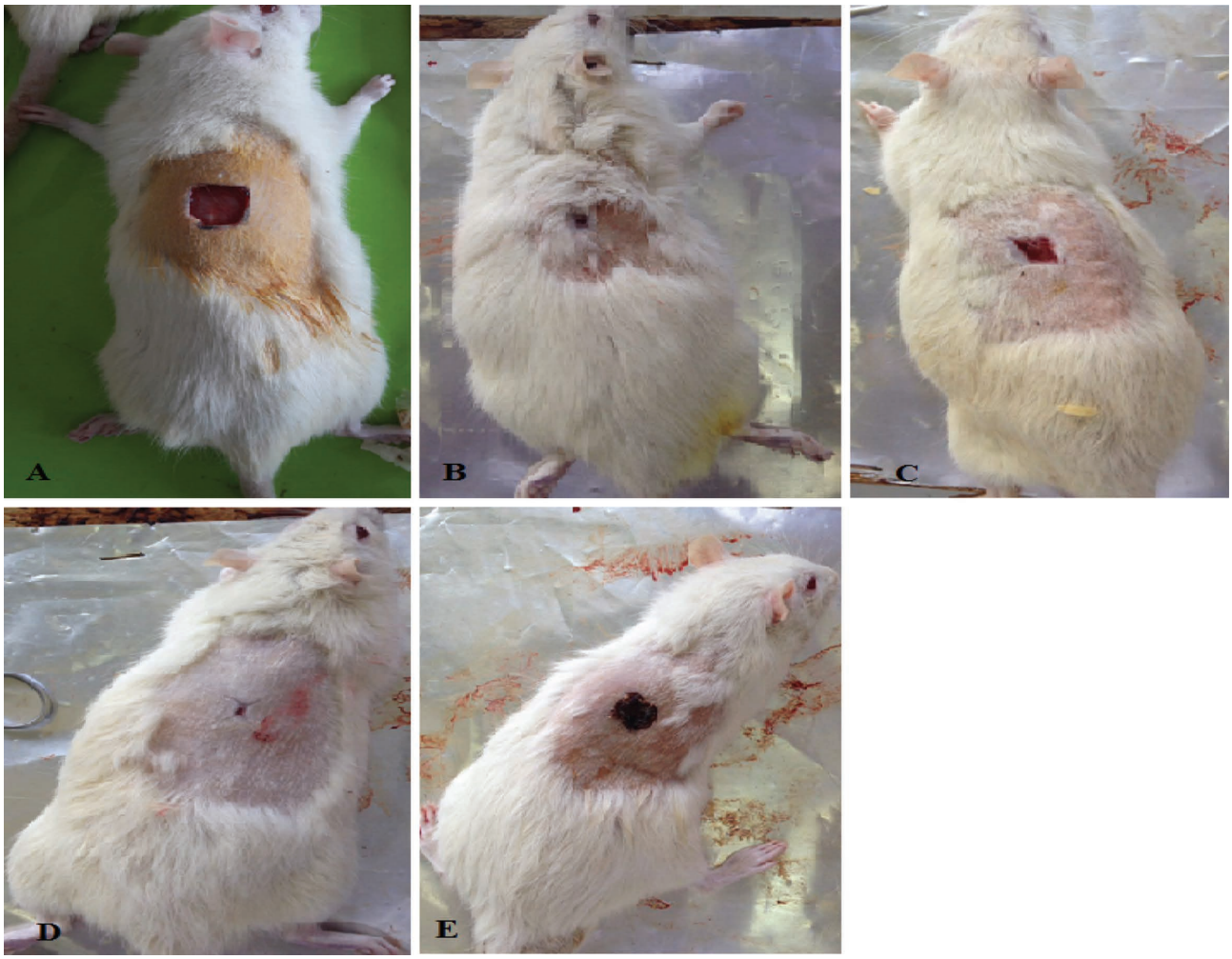


**Figure 2.** Photographic images of skin surface of mature male Wistar rats in control group on day 0 (A), day 15 (B), curcumin group on day 15 (C), fluorescent group on day 15 (D) and fluorescent and curcumin on day 15 (E).

 

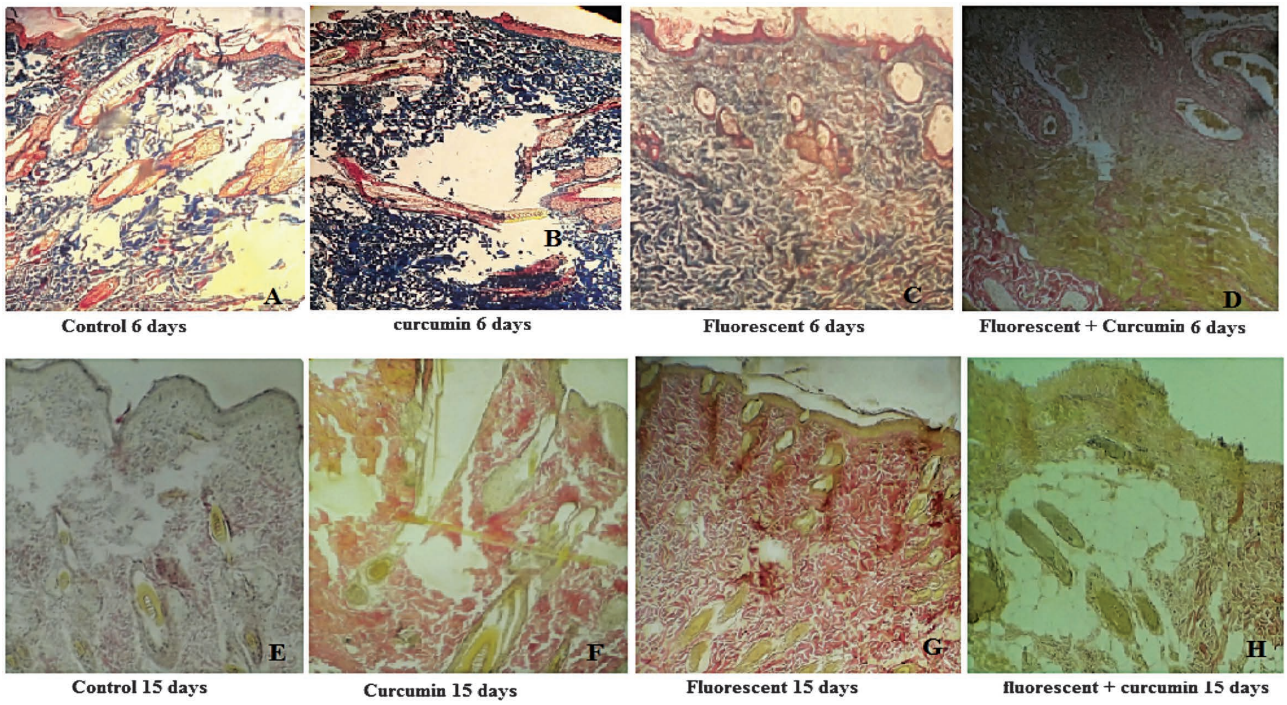


**Figure 3.** Skin morphology at the site of injury
Morphologic evaluation of injury site in mature male Wistar rats on days 6 and 15 in the control group (A & E), curcumin (B & F), fluorescent (C & G) and fluorescent plus curcumin (D & H) groups, respectively. H & E staining (A-D). PAS staining (E-H) with ×400 magnification.

of fewer glands and delayed improvement was observed in the curcumin plus fluorescent group (D).

On day 15, an increase was seen in the numbers of hair follicles in the control group, compared to the 6$^{th}$ day of the same group. In addition, the administration of curcumin resulted in enlarged hair follicles and sweat glands (E & F). In the fluorescent group, a substantial growth was observed in the number of hair follicles, and sweat and sebaceous glands were also observable (G). In fluorescent plus curcumin group, although the number of follicles increased from the 6$^{th}$ day up to day 15, the increase was less significant than that observed in other groups and fewer hair follicles were observed (H). Morphologic evaluations revealed that fluorescent light accelerated skin repair in Wistar rats (C & G).

## Serum findings

Average TNFα serum levels on day 6 showed a significant increase in the fluorescent group compared to controls and curcumin group (P<0.0001). However, TNFa levels on day 6 were significantly lower in curcumin plus fluorescent group when compared to the control, curcumin only and fluorescent only groups (P<0.0001, <0.003 and <0.0001, respectively). Average serum levels were, not significantly, lower in the curcumin group, (P=0.945). Average serum TNFα levels of curcumin plus fluorescent group on day 15 showed a significant decline compared to that of the control, curcumin only and fluorescent only groups (P<0.0001, 0.002 and <0.0001, respectively). TNFa levels of fluorescent group showed no significant alteration (P=0.945) (table 1).

**Table 1.** Mean and SDs of serum TNFα levels

| | Control | Curcumin (group A) | Fluorescent (group B) | Curcumin + fluorescent (group C) | P-value control vs group A | P-value control vs group B | P-value control vs group C |
|---|---|---|---|---|---|---|---|
| TNFα in 6$^{th}$ day | 15.3±0.65 | 14.95±0.69 | 17.1±0.76 | 13.7±0.46 | P=0.945 | P<0.0001 | P<0.0001 |
| TNFα in 15$^{th}$ day | 16.65±0.42 | 15.4±0.76 | 16.3±0.09 | 14.1±0.76 | P=0.003 | P=0.945 | P<0.0001 |

Serum TNFα levels of mature male Wistar rats from control, curcumin only, fluorescent only and fluorescent plus curcumin groups on days 0, 6 and 15. Data have been presented as mean±SD.

## DISCUSSION

The main objective of the study was to assess the effect of compressed fluorescent light exposure and curcumin administration on skin repair in mature male Wistar rats. Fluorescent light and curcumin were used separately and concomitantly to treat the injuries inflicted on the skin of the subjects.

Results of this study revealed that the use of fluorescent light can speed the process of healing when compared to the results of control, curcumin only and curcumin plus fluorescent light.

Recent studies have revealed that UV light may improve wound healing and re-establish skin homeostasis, alongside having anti-oxidative and anti-inflammatory attributes [11,12], which is in line with the present study, where UV light enhanced wound healing. It appears that compact fluorescent light leads to the modulation of certain chemoreceptors on super-cellular or subcellular levels, regulating cascade reactions within cells, thereby promoting wound healing. Wound healing, in essence, is a complex phenomenon in which many factors, cells, cytokines, mediators and ECM play a role [15].

The reduction in the wound size is considered to be an acceptable measure for wound healing. As healing progresses, the wound surface area reduces due to connective tissue formation. Fibroblasts induce contraction in the epidermis, closing the wound and decreasing its size [16].

Wound healing is comprised of four main stages which overlap at certain points, rendering it impossible to distinguish one from another [17]. By enhancing or speeding each of these stages, regardless of the qualitative or quantitative nature of the improvement, the overall healing process may be improved. Producing ECM and collagen, fibroblasts play an important role in wound healing. Myofibroblast activity is increased following UV light stimulation [15]. In addition, by improving blood flow and increasing vessel diameter, UV light improves the functional capacity of fibroblasts, conducing to the strength of the tissue by increasing collagen content [18].

Research has also revealed that low intensity electric stimulation promotes epithelial, fibroblast, neutrophil and macrophage migration, leading to a rapid resolve in the inflammatory phase and wound healing [19]. It is also speculated that UV light aids healing by inhibiting bacterial growth and keeping the surface sterile and dry. Similarly, it has been observed that administering antibiotics promotes the wound healing resulted from the inhibition of infectious overgrowth of the wound [20].

In our study, it was observed that groups receiving intraperitoneal curcumin underwent limited wound healing when compared to the control group, particularly on the 15$^{th}$ day. However, other studies have shown curcumin to be beneficial for wound healing [21]. In a study performed by Salehi et al., wound healing was found to be relatively similar in the control group and the group receiving systemic curcumin. In addition, curcumin administration improved wound healing in diabetic subjects [22]. It has been reported that local administration of curcumin promotes wound healing. In a study comparing the administration of curcumin (2%) with antibiotics such as silver sulfadiazine, it was reported that treatment with curcumin held better results compared to that of antibiotics [21]. It seems that the antibacterial and antifungal properties of local curcumin have a major role in the production of such results [23].

Curcumin accelerates diabetic wound healing because it has anti-inflammatory properties, prolonging monocyte survival, stimulating collagen production, reducing PGE2 levels, increasing cell wall fluidity, decreasing blood viscosity, and inhibiting platelet aggregation [24]. Tannin promotes wound contraction, increases epithelialization, capillary reformation and fibroblast proliferation [25].

Given the anti-inflammatory properties of curcumin and the fact that inflammation is an inseparable component of wound healing and that the presence of cells and catabolic cytokines such as interleukins, anabolic cytokines such as growth factors, are integrated into this stage, the inhibition of inflammation entails no benefits and has negative effects on wound healing [5,26].

However, a number of reports have evidenced the positive impact of both local and systemic curcumin administration on wound healing. In addition, a number of studies have reported that curcumin has no beneficial impact on wound healing. Negative results obtained in our study may be attributed to the high dosage of curcumin administered; at high doses, curcumin promotes cell apoptosis in fibroblasts. A study performed by Scharstahl et al. reported that a dosage of 25

micromoles of curcumin led to apoptosis in the fibroblasts [27].

In the current study a significant wound healing was observed on day 6 by simultaneous treatment with compact fluorescent light and curcumin, when compared to controls. However, a significant decrease was observed on the 15th day, when compared to other groups. It was further observed that fluorescent light is able to promote wound healing, while mere treatment with curcumin lacks such attribute. Since high dosages of curcumin inhibit fibroblasts and cell proliferation, healing effects up to the 6th day are solely attributed to that of the fluorescent light. However, on day 15, it was revealed that both treatments had negative effects on wound healing since subjects showed a marked weight reduction when compared to other groups. This may be due to the widespread infection, increased apoptosis and delayed wound healing during the 15-day period.

In the current study, a reduction was observed in TNFα levels in groups receiving curcumin . One of the many attributes of curcumin is its anti-inflammatory properties, demonstrated in our study. Curcumin prevents the transcription of many genes such as those coding inflammatory cytokines and enzymes [28,29]. In addition, the inhibition of NF-kB halts the production of cyclooxygenase 2, IL-1, IL-6, IL-8, TNFa and various other chemokines. Therefore, the findings of our study are consistent with that of studies demonstrating a reduced level of TNFα in groups receiving curcumin [30,31]. However, simultaneous treatment with curcumin and fluorescent light was hazardous to the healing process, hence spreading infection and inducing weight loss in the subjects.

## CONCLUSION

Fluorescent therapy was shown to promote wound healing when compared to other groups (curcumin only, fluorescent plus curcumin, and controls). Such results are attributable to increased blood flow, regulated homeostasis, inflammation, and prevention of infections. Negative results obtained in the group treated solely with curcumin may be due to the excessive doses administered; at high doses, the anti-inflammatory attributes of curcumin inhibit cytokine production, hence promoting infections, both locally and systemically.

We suggest that future studies focus on the mechanisms through which fluorescent light promotes wound healing. Moreover, assessing the impact of different doses of curcumin on wound healing may prove to be of benefit.

**Conflict of Interest:** None declared.

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