

## Effect of *Curcuma longa* Crude Extract, Curcumin and Nano Curcumin-based Gel Topical Administration on Excised Skin Wound Healing in Swiss-Webster Mice

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Patients suffering from skin wounds often have their life heavily affected economically and socially. In general, wound healing consists of four different sequentially and overlapping phases. Interrupting those phases results in extending the healing time. Turmeric (*Curcuma longa*) is widely used by Indonesians to treat wounds, with curcumin as an active substance, which is proven to have various effects on improving wound healing. Therefore, this study aims to determine how the topical application of turmeric gel on affects the acceleration of wound closure. Analytical laboratory experiments were conducted on 24 male Swiss-Webster mice. Subjects were divided into four groups according to where the excision wounds were made on the back, and observed on days 0, 3, 6, 9, 12, and 15 post wound induction. The wound healing acceleration was observed after 3 ( $p = 0.0003$ ), 6 ( $p = 0.0085$ ), and 9 ( $p = 0.0023$ ) days in *C. longa*-treated mice compared to the control group. However, there was no significant difference found on days 12 and 15 after wounding. In addition, the area under the curve analysis shows significant difference between the *C. longa*-treated group compared to the control, but not with the group treated with neither curcumin nor nano curcumin gel. In conclusion, topical application of 3% *C. longa* extract gel improves excised skin healing.

**Keywords:** *Curcuma Longa* Gel; Curcumin Gel; Excision Wound; Nano Curcumin Gel; Wound Healing Acceleration.

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Skin wounds have enormous social and economic implications, but the significance of wound research seems to be overlooked because studies have mostly focused on the disease that caused them. Patients with surgical and traumatic wounds, abrasion, and superficial burns are

characterised by the occurrence of acute wound.<sup>1</sup> Wound healing involves four distinct overlapping phases that must occur at a specific and precise times. Several diseases such Diabetes causes abnormal wound healing because of complex mechanisms that eventually lead to chronic

wounds.<sup>2</sup> Functional impairment due to chronic wounds causes a decrease in quality of life and a high financial burden for wound care and treatment.<sup>3</sup> Therefore, it is necessary to study the factors that induce chronic wounds as well as any potential accelerator of wound healing time.

Plants and their compounds have potential for wound management and treatment. Medicinal plants are not only cheap and affordable, but also believed to be safe for use with rarely encountered hypersensitive reactions.<sup>4</sup> *Curcuma longa* or turmeric is a biopharmaceutical plant highly produced in Indonesia. Locals have been using this plant as ingredient in traditional foods and herbs to resolve tissue inflammation due to its anti-inflammatory, anti-infectious, and antioxidant active substance content. *Curcuma longa* has undoubtedly the potential to act as a wound healing agent.<sup>5,6</sup>

On the other hand, its active compound, *curcumin* has been developed in a new nano size form that shows improvement on bioavailability after consumed.<sup>7</sup> We therefore conducted a study of excised skin wound healing using *C. longa* administration to examine the impact on wound healing.

## MATERIALS AND METHODS

Experimental *in vivo* laboratory analytical research was conducted on 48 male mice (*Mus musculus*) strain Swiss-Webster aged 8-10 weeks and weighing 22–26 g. The mice were obtained from the Pharmacology Laboratory, Faculty of Medicine, Universitas Padjajaran.

### Production of topical gel

Blank gel was produced using pure gel that was free of any active ingredients. *Curcuma longa* extract gel was made by combining 96% v/v ethanol extract of *C. longa* with gel (0.3%). The gels were developed in the Pharmacognosy Laboratory at Padjadjaran University's Faculty of Pharmacy. Curcumin (0.25% w/v) was mixed into a traditional emulsion with GMO (16 w/v) and Chemophor RH40 to make nanocurcumin gel. The curcumin nanoemulsion was then processed into a gel. This gel was produced in the Semisolid Laboratory of the Faculty of Pharmacy at the Bandung Institute of Technology (ITB). For this experiment, each gel

contained 3% *C. longa* extract, 3% curcumin, and 3% nanocurcumin respectively.

### Wound induction

Before the experiment, the mice were acclimatised for seven days and weighed. They were housed in regular cages with free access to food and water.

Following the adaptation phase, the body weight was measured again, and elimination was carried out in accordance with the exclusion criteria.

Before wounding, the hair on the back of each mouse was shaved and sterilized. An isofluran inhalation was used to provide anaesthesia before two excision wounds were created. Alcohol 70% was used for aseptic and antiseptic procedures on the wound area. Skin excision was performed using a 6 mm punch biopsy (GlaxoSmithKline GmbH & Co. KG, Germany) to ensure homogenous depth.<sup>8,9</sup>

### Intervention

The experimental animals were divided into four groups, including a control (n = 6) treated with blank gel daily, and three treatment groups treated with turmeric extract gel (n = 6), curcumin gel (n = 6), and nanocurcumin gel (n = 6) topically on the wound surface every day.

### Wound Area Analysis

The wound area was observed to determine the effect of turmeric gel on the percentage of skin coverage of the excision wound. Observations were made on the percentage of mice skin wound closure in each treatment group on days 3, 6, 9, 12 and 15 after wound induction. The area of the wound was measured using the Image J application (open source), while initial wound closure was defined as 0% closure.<sup>8,9</sup>

$$\% \text{ wound closure} = (\text{Initial wound area} - \text{final wound area}) \times 100\% / (\text{Initial wound area})$$

### Statistical Analysis

Statistical analysis and graph processing were performed using Graph Pad Prism 8. Normality and homogeneity test of wound closure percentage was carried out using Saphira-Wilk and Levene, respectively. The data with normal and abnormal distribution were analysed using one way-ANOVA and Kruskal-Wallis, respectively, to compare the results of the treatments.

### Ethical clearance

The experiment in this study has followed ethical standards in animals, and has received a statement of ethical eligibility from the Research Ethics Committee of the Faculty of Medicine, Universitas Padjajaran with number 705/UN6.C.10/PN/ 2017.

### RESULTS

Administration of topical *Curcuma longa* gel accelerated wound closure on days 3, 6, and 9 post wound induction

The wound closure percentage shows that administration of topical gel accelerated wound closure on days 3, 6, and 9 post wound induction ( $p = 0.0003$ ,  $p = 0.0085$  and  $p = 0.0023$ , respectively). However, on days 12 and 15 post wound induction, there is no significant differences among the groups (Figure 1).

As shown in Figure 1, topical administration of *C. longa* gel increases the speed of wound closure on days 3, 6, and 9 after induction. The Area Under the Curve (AUC) analysis (Figures 2 and 3) shows that there was a significant difference in the speed of wound closure in the four groups ( $p < 0.05$ ), more specifically, only the group given *C. longa* gel has a significant difference compared to the control group ( $p < 0.05$ ). This is demonstrated by the higher mean AUC of

347.7 in the process of wound closure compared to 289.6 of the control group (Figure 3). Thus, topical *C. longa* administration significantly accelerates the closure of the excision skin wounds.

In the observation, *Curcumin* gel only showed an increasing wound closure effect on day 9 ( $p < 0.05$ ), whereas the nanocurcumin treated group did not improve wound closure. Although there is evidence of wound healing acceleration (Figure 3), no significant differences in the AUC of the curcumin gel and nanocurcumin gel groups and the control group were found. Macroscopic features of each group are presented in Figure 4.

### DISCUSSION

In general, the four healing phases occur 2-3 weeks post wounding.<sup>10</sup> The first phase, coagulation, is required for haemostasis and wound protection.<sup>11</sup> The clot and the surrounding wound tissue will release pro-inflammatory cytokines and growth factors. Once bleeding is controlled, inflammatory cells migrate to the wound area (chemotaxis) and initiate an inflammatory phase, which is characterised by sequential infiltration of neutrophils, macrophages, and lymphocytes.<sup>12</sup>

After arriving at the wound area, polymorph nuclear leucocyte (PMN) phagocytes bacteria and other foreign particles in the wound area. Blood monocytes undergo phenotypic changes

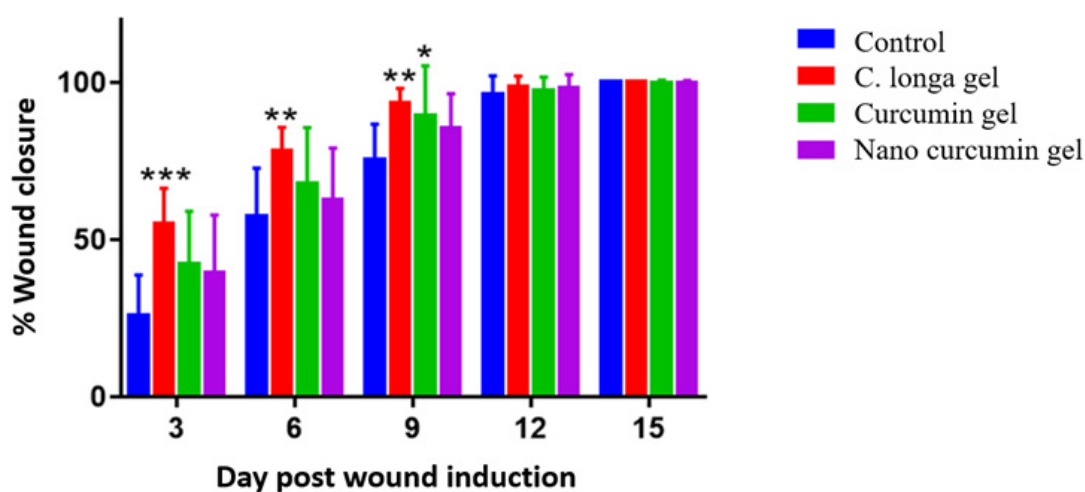


Fig. 1. Wound closure was observed in the control group, *Curcuma longa*-treated mice, and nanocurcumin-treated mice on days 3, 6, 9, 12, 15, 18, 21, and 24 after wound induction

when they are in the wound area by becoming M1 macrophages, which are pro-inflammatory. Macrophages cells have the most important role of releasing proteolytic enzymes such as collagenase to clean tissue. After cleaning apoptotic cells, macrophages undergo phenotypic changes into M2 macrophages, which are anti-inflammatory and stimulate keratinocytes, fibroblasts, and angiogenesis needed in tissue regeneration and proliferation.<sup>12</sup> Lymphocytes are the last cell type to enter wound blood during the inflammatory

phase and produce various interleukins (IL). One of which is IL-1, believed to play a key role in collagenase regulation, collagen repair, and extracellular matrix (ECM). IL-4 and IL-13, which are also produced by lymphocytes, act as inhibitors in M1 activation and trigger M2 activation.<sup>13</sup>

The proliferation phase is characterised by the proliferation and migration of epithelial cells to a temporary matrix in the wound area. In this phase, granulomatous tissue formation occurs to restore the fibrin matrix and as a new pathway

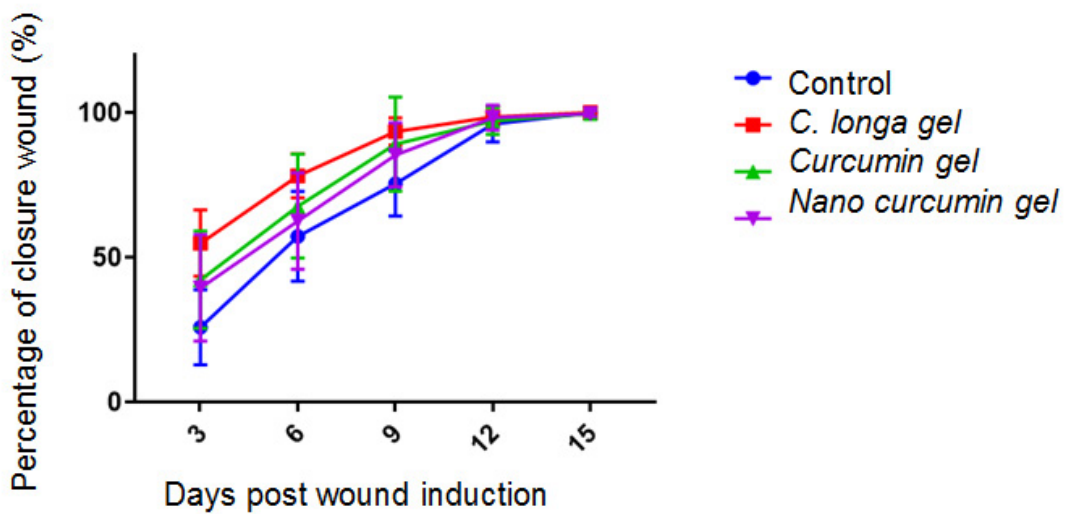


Fig. 2. The wound closure development %

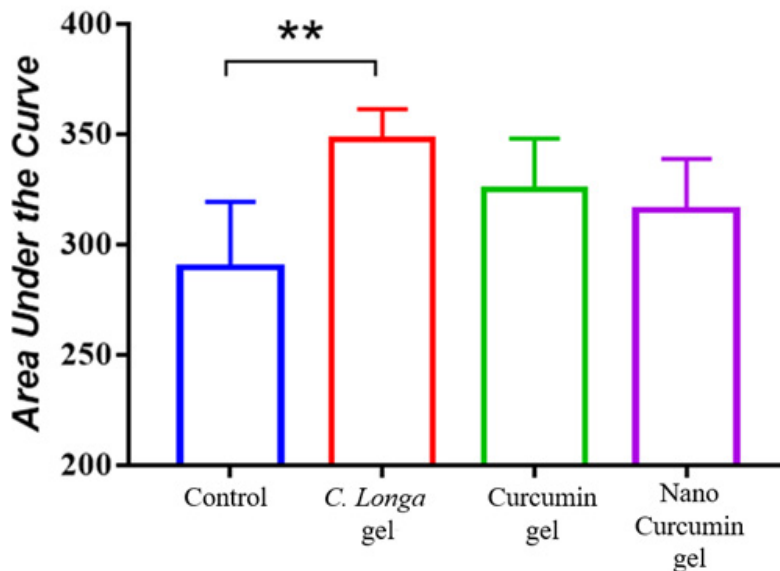


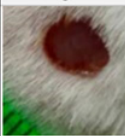
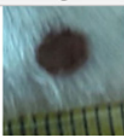
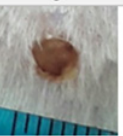
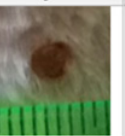
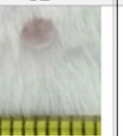
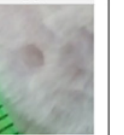



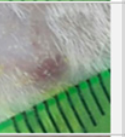
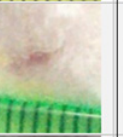

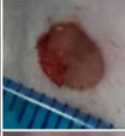
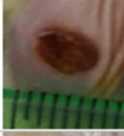
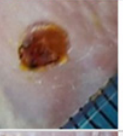
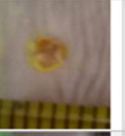
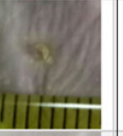

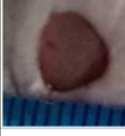
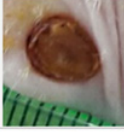
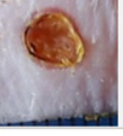
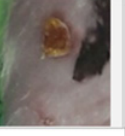
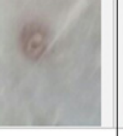

Fig. 3. Area Under the Curve of all treatments

for keratinocyte migration.<sup>10,12</sup> Keratinocytes play a role in restoring the protective function of the epithelium. In the final proliferation phase, some of the fibroblasts may differentiate into myofibroblasts, which contract quickly and efficiently to close the wound. The whole healing process stops when the last phase, remodelling, takes place. The interaction of fibroblasts and myofibroblasts produces an extracellular matrix, especially in the form of collagen.<sup>10,11</sup> An important feature of the remodelling phase is that there are many new capillary regressions that will restore the density of blood vessels in the wound tissue back to normal and ECM repair with an architecture close to normal tissue.<sup>12</sup>

A number of studies have been conducted to explore *curcumin*'s ability to heal cutaneous<sup>14-17</sup> and excision wounds.<sup>18,19,20,21,22</sup> The role of *curcumin* in promoting epithelial regeneration, fibroblast proliferation, and vascular density has been explored in various studies.<sup>16,22</sup> *Curcumin* has been shown to increase IL-4 and IL-13, up-regulation of mRNA, and induce M2 polarisation so as to accelerate the proliferation phase of wound healing.<sup>23</sup> During the remodelling phase, *curcumin* has a role in increasing granulomatous tissue formation,<sup>14</sup> repair, collagen deposition,<sup>19,24</sup>

wound contractions, and many positive effects on the proliferation and remodelling phase.<sup>18,24,25</sup> However, due to its anti-inflammatory properties, *curcumin* administration also has a negative effect on the inflammatory phase. *Curcumin* has an inhibitory function (nuclear factor kappa-light-chain-enhancer of activated B cells) NF- $\kappa$ B and cyclooxygenase-2 (COX-2), inhibits arachidonic acid metabolism, decreases the expression of cytokines IL-1b, IL-6, (tumour necrosis factor alpha) TNF- $\alpha$ , and down-regulation enzymes such as protein kinase C. Experiments on *curcumin* administration in macrophage cultures showed inhibition of M1 polarisation, TNF-a production, NF- $\kappa$ B phosphorylation, and decreased TLR4 (toll-like receptor 4) protein expression, which is a regulator of macrophage polarisation M1.<sup>23,26,27</sup> Macrophage function, as the main regulator of the inflammatory phase, is disrupted due to the effect of *curcumin*. Considering that the slightest interruption in the overlapping wound healing phase can cause delayed healing, it is necessary to further study *curcumin* administration during days 3-4 after wound induction when the inflammatory phase of has been completed.<sup>28</sup>

As commonly known, besides *curcumin*, there are other active substances in *C. longa*

| Treatment cohorts    | The Wound Comparison Post Induction   |   |   |  |   |   |
|----------------------|---|---|---|--|---|---|
|                      | 0   | 3   | 6   | 9  | 12  | 15  |
| Control              |  |  |  |  |  |  |
| <i>C. longa</i>      |  |  |  |  |  |  |
| <i>Curcumin</i>      |  |  |  |  |  |  |
| <i>Nano curcumin</i> |  |  |  |  |  |  |

**Fig. 4.** Macroscopic appearance of wound closure in each group on day 0, 6, 9, 12, and 15 post wound induction

which have been shown to have a positive effect on the speed of healing wounds. Two other curcuminoids, namely desmethoxycurcumin and bisdemethoxycurcumin, have the same effect as *curcumin*.<sup>2, 26,29</sup> The essential oil of *C. longa* has strong antibacterial and anti-inflammatory activity in addition to protein, fat, and vitamin, which have an important role in wound healing and regeneration.<sup>4,30</sup> This characteristic may be the driving effect of accelerating wound closure compared to *curcumin* or *nanocurcumin* gel alone. To better understand the effects of the various substances contained in turmeric, further investigation is thus required.

One of the limitations of this study that should be highlighted is the behaviour of experimental mice, which might have caused the unexpected results. The large number of mice placed in a single cage may affect the topical administration and reduce the effectiveness of the gel. Mice usually scratch, bite, or lick their or other mice's backs, affecting the gel applied. In addition, the number of experimental animals in this study was relatively small and less homogeneous, which might have cause the large variations of the resulting data.

### CONCLUSION

Our results of wound healing on days 3, 6, 9, 12, and 15 showed a positive effect on the acceleration of wound healing by *C. longa* gel. Although *curcumin* gel treatment and its nanoparticle form (*nano curcumin*) accelerated wound healing, the ability of both gels is still inconclusive and should be tested on a larger sample, at different concentrations and on different test subjects.

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### Conflict of Interests

Authors declare no conflict of interests.

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