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Topical curcumin gel accelerates healing of II A – degree burns on male wistar rats (*Rattus norvegicus*)

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ABSTRACT

Background: Burns can happen to anyone at any time, so they require wound care, which is essential to speed up wound healing. Curcumin gel is suspected to help this process, which is administered topically. Besides being cheap, easy to find, and minimal side effects, the content of curcumin helps a series of biological processes that accelerate the recovery of II A – degree burns, which have not been studied extensively recently.

Aim: The study aims to prove that topical curcumin gel can accelerate the healing of II A-degree burns in male Wistar rats (*Rattus norvegicus*).

Methods: This true experimental study with a post-test control group design involved 28 male Wistar rats (*Rattus norvegicus*), which were initially intervened by inducing a heat probe that stimulated the formation of an area of II A – degree burns. Wound profile analysis was conducted in the control and treatment groups within 7 and 14 days, respectively. After the wound biopsy, the significance of the parametric test results on the average velocity of epithelialization, number of fibroblasts, and collagen density was assessed.

Results: Epithelialization rate ($p<0.001$), fibroblast cell count ($p=0.007$), and collagen density ($p=0.011$) after curcumin administration were higher than controls. The curcumin treatment group at 14 days showed the greatest significance among the other comparisons ($p<0.05$). The higher the speed of epithelialization, the higher the number of fibroblasts or collagen, respectively, in the control and treatment groups ($r > 0.65$; $p <0.05$).

Conclusion: Topical administration of curcumin gel has the potential to be a hope for future remedies that initiate the improvement of the healing process of II A – degree burns properly.

Keywords: Burns, Collagen, Curcumin, Epithelialization, Fibroblasts, Paraffin gauze.
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INTRODUCTION

Second-degree A-B (shallow-deep) burns involve skin damage from the epidermal layer to some of the deeper dermis. These burns dominate the percentage of the highest incidence rate among other degrees, which is equal to 73%, while the incidence rate of first-degree burns is as much as 17%, and the remaining 10% are third-degree burns.[1] Burns cause local and systemic effects that are very complex for the body. Local effects occur in the skin and subcutaneous tissue, while systemic effects affect capillaries, metabolic processes, body temperature control, the immune system, and the function of the kidneys and lungs. The magnitude of the effect depends on the depth and extent of the tissue damaged by the burn. One of the efforts that can be made to accelerate the healing of burns is to treat the wound.[2]

Treatment of wounds in cases of burns is adjusted to the degree, for burns of II B - degree (deep) to III - degree tend to require surgery such as tangential excision, skin graft or flap. Wound care can be done conventionally for burns of I to II A - degree (shallow), including by irrigation using 0.9% NaCl or applying topical agents such as Paraffin Gauze. However, this method often causes dry wounds, causing pain and discomfort and disrupting the formation of new epithelium. In addition, conventional techniques are more susceptible to bacterial infection and difficult to absorb exudate.[2] There are modern wound care methods, but they often require quite high costs. Based on this, studies on alternative methods of healing burns using various medicinal plants have begun to be developed.[3]

Turmeric is a rhizome perennial herb from the ginger family Zingiberaceae. The most important chemical component of turmeric is a group of compounds called curcuminoids or curcumin (77% diferuloylmethane, 17% demethoxycurcumin, and 3% bisdemethoxycurcumin). The diferuloylmethane structure of curcumin

is responsible for its antioxidant, anti-inflammatory, anti-allergic, anti-carcinogenic, anti-mutagenic, anti-coagulant, anti-bacterial, anti-fungal and anti-septic effects. The potential efficacy of curcumin is mainly in oral form, but its effectiveness is constrained by its hydrophobic nature, so its absorption becomes less good. Meanwhile, topical curcumin can be easily formulated to increase penetration into the skin, and absorption is not affected by the stomach and can be improved, especially when inflammation occurs, or the skin barrier is damaged due to trauma or certain diseases.[4]

There is a greater inflammatory response than other wounds in the case of burns, so curcumin is very attractive for use in healing burns.[5] A Research in 2021 has proved a histological picture of faster healing of burns in experimental rats given curcumin gel compared to the control group.[6] As a consideration of the administration dose, the content of 2% curcumin gel is effective in healing periodontitis in Wistar rats.[7] In the case of burns, the inflammatory reaction is not entirely worse, so the content of 1% curcumin gel is expected to be the right dosage choice for healing burns. Meanwhile, no study proves that a topical gel containing 1% curcumin can accelerate the healing of burns, specifically in II A - degree, compared to conventional methods in Wistar rats. Therefore, this study aims to prove that topical curcumin gel usage can accelerate the healing process of IIA-degree burns in Wistar rats compared to conventional methods using Paraffin Gauze macroscopically and microscopically.

METHODS

This research was a true experimental study with a post-test control group design, which was conducted at the Integrated Laboratory Unit of the Faculty of Medicine, Udayana University, where the rats were treated and, simultaneously, the process of taking their tissue was carried out. This study was conducted for 3 months, from November 2022 to January 2023. The samples in this study were adult rats that met the following inclusion criteria: male Wistar rats (*Rattus norvegicus),* aged 8-12 weeks (2-3 months), and body weight 200-250 grams.

Twenty-eight rats had been acclimatized for 7 days, randomly grouped into 4 groups and placed in the cages provided. Label P1 is a treatment to assess epithelialization, fibroblast count and collagen density on day 7; P2 is a treatment to evaluate epithelialization, fibroblast count and collagen density on day 14; K1 is a control to assess epithelialization, fibroblast count and collagen density on day 7; and K2 which was a control to assess epithelialization, fibroblast count and collagen density on day 14.

The II-A burns caused injury to the back of the Wistar rat by attaching an iron metal 2 cm in diameter that had been dipped in hot water ± 90-100°C for 10 minutes. Iron metal was attached to the wound area for 20 seconds, washed with sterile water and dried until an II-A burn was formed, characterized by a reddish color and bullae (water bubbles) on the rat's skin.

Curcumin powder 100 g obtained from Bali Pure Home Industry® is 100% turmeric powder from selected local farmers in Bali. The powdered curcumin was then processed at the Pharmacology Laboratory of Mahaganesha University Denpasar to obtain topical curcumin gel with a concentration of 1%. Then it was applied to the second-degree burns of Witsar rats with a diameter of 2 cm and covered with a transparent dressing.

Paraffin gauze or tulle is a dressing in the form of a sheet-like gauze with holes that are rarer but stronger, does not leave pieces of cloth/thread in the wound and has a relatively fixed shape (unlike gauze). Processed sterilely, applied to second-degree burns of Wistar rats with a diameter of 2 cm and covered with a transparent dressing.

Fibroblast cells were counted with histopathological characteristics in the form of squamous cells with reddish cytoplasmic projections, oval nuclei with little chromatin, and one or two nuclei in the wound, which were prepared with hematoxylin-eosin (HE) staining at 400x magnification. Digital analysis using an Olympus CX21 microscope (Japan), photographed with an optilab Pro camera (Miconos, Indonesia) by an Anatomical Pathology Specialist at the Denpasar Central Laboratory.

Bright red fibers produced by fibroblast cells in the wound which were prepared by staining with Picro Sirius red at 400x magnification. Digital analysis using an Olympus CX21 microscope (Japan), photographed with an optilabPro camera (Miconos, Indonesia) by an Anatomical Pathology Specialist at the Denpasar Central Laboratory. Clinically characterized wound healing process, i.e., the lesions are pink, dry and not attached to the dressing. Epithelialization assessment was carried out by displaying clinical photos of the wound from the first day until the last day before the biopsy. The images were then processed using the ImageJ application, measuring threshold and pixel changes at the wound's edges. Epithelialization can be calculated using the Bloemen formula: 1 – (pixel epithelial area/pixel total wound area) × 100%, then the average value of each treatment group is calculated.

On days 7 and 14, all rats treated with sedation were as follows, as explained above. Remove the transparent dressing slowly, then burn tissue is taken along with 1-2 mm of healthy tissue around the wound.[8] Subsequent plots followed histotechnical guidance. The skin sample was fixed in 10% Neutral Buffer Formalin (NBF) solution and then sent using a container to the Denpasar Sentra Laboratory and processed to make preparations until ready for examination under a microscope. HE staining was used to assess the amount of collagen, and Pircosirius Red staining was used to determine collagen density.

Euthanasia was carried out 4 weeks post-action after the sample harvesting process with intracardiac administration of Ketamine (30 mg/head) and high-dose Xylazine (6mg/head). The experimental animals were then put into the incinerator.

Data analysis in this study consisted of descriptive statistical analysis, normality test, variance homogeneity test and comparison test (hypothesis test). The hypothesis test/comparison aims to compare the speed of the epithelialization process between groups and the amount of angiogenesis, fibroblasts and collagen deposition between groups and over time.

RESULTS

Based on Figure 1, the reduction in the burn area compared to its previous size indicates a healing process through the growth of supporting tissue, including epithelialization, which is easy to see without a microscope. However, epithelialization that occasionally occurs, especially on day 3 and after pre-biopsy is performed, is examined by perpendicular photography over the radius of the burn area with image pixels and wound area pixels defined in ImageJ. Changing the image threshold and focusing on the wound area makes it easier to calculate the speed of epithelialization.

Based on Table 1, epithelialization is expressed by Bloemen's formula, in which ImageJ has determined the pixel value of the wound area. Bloemen's formula produces a number in percentage form by focusing on the total wound area compared to the entire image area. The difference in the percentage increase in epithelialization was greatest in the P2 group compared to K2. The P1 group also displayed a greater increase in the percentage of epithelialization compared to K1.

The variable number of fibroblasts was obtained from the number of fibroblasts observed in histological preparations with HE staining viewed at 400 times magnification (Figure 2) under an Olympus CX21 trinocular microscope (Japan), Optilab Pro camera (Miconos, Indonesia), as well as the Optilab Viewer2.2 software (Miconos, Indonesia) with PC Imaging Software Windows OS EP50 at 5 lpb. The results of calculating the number of fibroblasts can be seen in Table 2.

Variable collagen density was obtained from the thickness of collagen observed in histological preparations with picrosirius red staining viewed at 400 times magnification under a trinocular microscope Olympus CX21 (Japan), Optilab Pro camera (Miconos, Indonesia), and Optilab Viewer2.2 software (Miconos), Indonesia) with PC Imaging Software Windows OS EP50 at 5 fov. Collagen density is calculated as the percentage of pixels where the collagen area *minus* the non-collagen area (Table 3). The red color channel separates the collagen image through the "RGBstack" function in Image J. Then it is changed to black and white, and only the dermis is blocked (Figure 3) so that the epidermis and subcutaneous tissue are "excluded" (you can see a yellow line) then in "adjust" a "threshold" value is made for the black and white color zone (the white area is non-collagen and the black space is collagen).

The three study variables found that the average was higher in the treatment group compared to the control group, both on the 7th and 14th days. The average difference recorded from calculating histological preparations of Wistar rat skin specimens under a trinocular microscope was then tested for statistical comparisons related to the significance of the mean differences between groups.

From the normality test results with the Shapiro-Wilk test, epithelialization data, fibroblast count, and collagen density in each control and treatment group on days

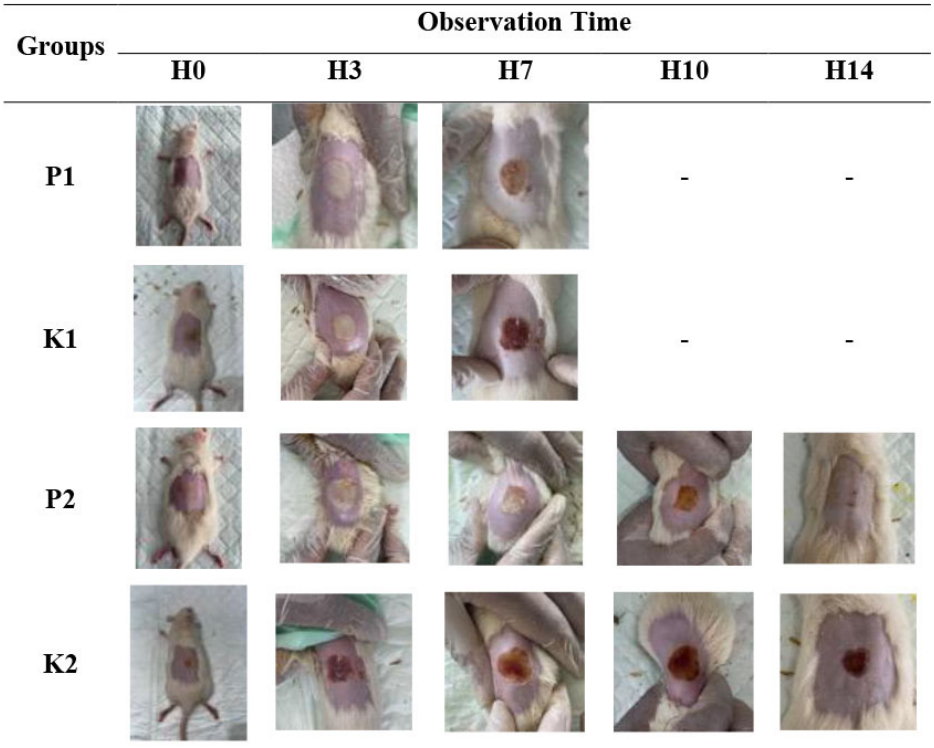

Figure 1. Observation of the description of second-degree burns in the treatment and control groups from day-0 to the pre-biopsy time.

Table 1. Epithelialization on day 3 to pre-biopsy day

Percentage of epithelial areas that appear in the post-burn area (%)				
	K1	K2	P1	P2
3rd day	64.3	47.3	65.9	72.0
	64.4	65.9	64.9	63.9
	60.0	60.3	69.0	61.2
	67.0	57.1	55.7	57.8
	54.0	44.9	59.5	54.6
	56.2	69.3	63.9	70.3
	61.4	56.4	51.5	54.0
Pre-biopsy (7th or 14th day)	63.6	80.2	65.9	87.6
	68.1	80.6	61.7	96.0
	61.9	63.0	62.3	87.6
	59.9	63.7	65.2	86.4
	64.5	89.6	68.1	77.1
	65.6	78.3	67.3	78.2
	59.2	75.3	70.1	81.9

Table 2. Number of Fibroblasts per 5 visual fields on days 7 and 14

The average number of fibroblasts per 5 fields of view			
7th day		14th day	
K1	9.3	K2	21
	15.3		18
	8.3		28
	8.6		42
	12.6		17.6
P1	1	P2	23
	7		35.7
	40		55
	32		38
	11.3		38.3
	24.3		39.7
	23		34.7
	16		27.7
	20		27.3

Table 3. Percentage of collagen density after the biopsy on days 7 and 14

Percentage of collagen density per 5 fields of view (%)			
7th day		14th day	
K1	3.933	K2	40.221
	21.284		60.119
	4.746		50.961
	7.263		49.669
	11.338		29.118
P1	14.679	P2	51.378
	5.9		44.205
	34.809		69.969
	38.066		68.236
	32.103		70.669
	31.026		70.119
	24.896		66.917
	30.875		72.801
	28.986		81.206

Table 4. Paired t-test of epithelialization rate

Groups	Epithelialization (%)	Mean ± SD	t	P
K1	Early day	61.04 ± 4.69	-0.932	0.38
	Pre-biopsy	63.25 ± 3.17		
P1	Early day	61.48 ± 6.19	-1.32	0.23
	Pre-biopsy	65.80 ± 3.04		
K2	Early day	57.31 ± 8. 95	-3.21	0.02*
	Pre-biopsy	75.82 ± 9.57		
P2	Early day	61.97 ± 7.18	-7.16	0.00*
	Pre-biopsy	84.98 ± 6.52		

Description: *Significance of paired t-test (p<0.05)

7 and 14 were normally distributed (p > 0.05). The variance of the epithelialization variable data, the number of fibroblasts, and the density of collagen in the treatment and control groups on days 7 and 14 were homogeneous (p ≥ 0.05) so hypothesis testing was continued with a parametric independent t-test (equal variance).

Calculation of the quantitative analysis of the speed of epithelialization based on the difference in the size of the second-degree burn area from the beginning of observation to the last day before the wound biopsy test can be seen in Table 5. In all groups, both control and treatment, which had received initial intervention in a hot sonde, the burn area appeared clear with computerized counting results (pixel scale). Between the initial observation day and the final day before wound biopsy, only the K2 and P2 groups showed a significant difference in average increase in epithelialization (p <0.05).

The average rate of epithelialization between the control and treatment groups was statistically different on day 14 (p <0.05). However, suppose the data includes all controls and treatments (four groups). In that case, the speed of epithelialization shows a significant comparison between the final and initial processes of observing wound specimens (Table 5).

Comparative analysis of the number of fibroblasts in the treatment group and the control group can be seen in Table 6. The analysis using the independent t-test showed a significantly higher average in the treatment group compared to the control group, with an average difference of 12.85 (p = 0.007; CI 95% = 3.91 – 21.79). The results of the analysis in Table 6 also include a higher average difference in the P1 group, which is significant compared to the K1 group with an average difference of 14.94 (p = 0.003; 95% CI 6.14 – 23.72) as well as between groups P2 and K2 are significantly different with an average difference of 10.77 (p = 0.05; 95% CI = 0.07 – 21.62).

Comparative analysis with the independent t-test for the collagen density variable on days 7 and 14 can be seen in Table 7. Collagen density on day 7 was significantly higher in group P1 than in group K1, with an average difference of 21.66 (p < 0.001; 95% CI = 15.42 – 27.90). Collagen density on day 14 was higher in group P2 than in group K2 (p <0.001; 95% CI = 15.89 – 28.83), with a mean difference in collagen density of 22.36. Generally, Table 7 explains that the average in the treatment group is significantly higher than the control group, with an average difference of 22.00 (p = 0.011; 95% CI = 5.56 – 38.46).

Based on the multivariate analysis in Table 8, the three research variables show results mutually correlated (p <0.05). The r-value or the correlation coefficient for each relationship between variables

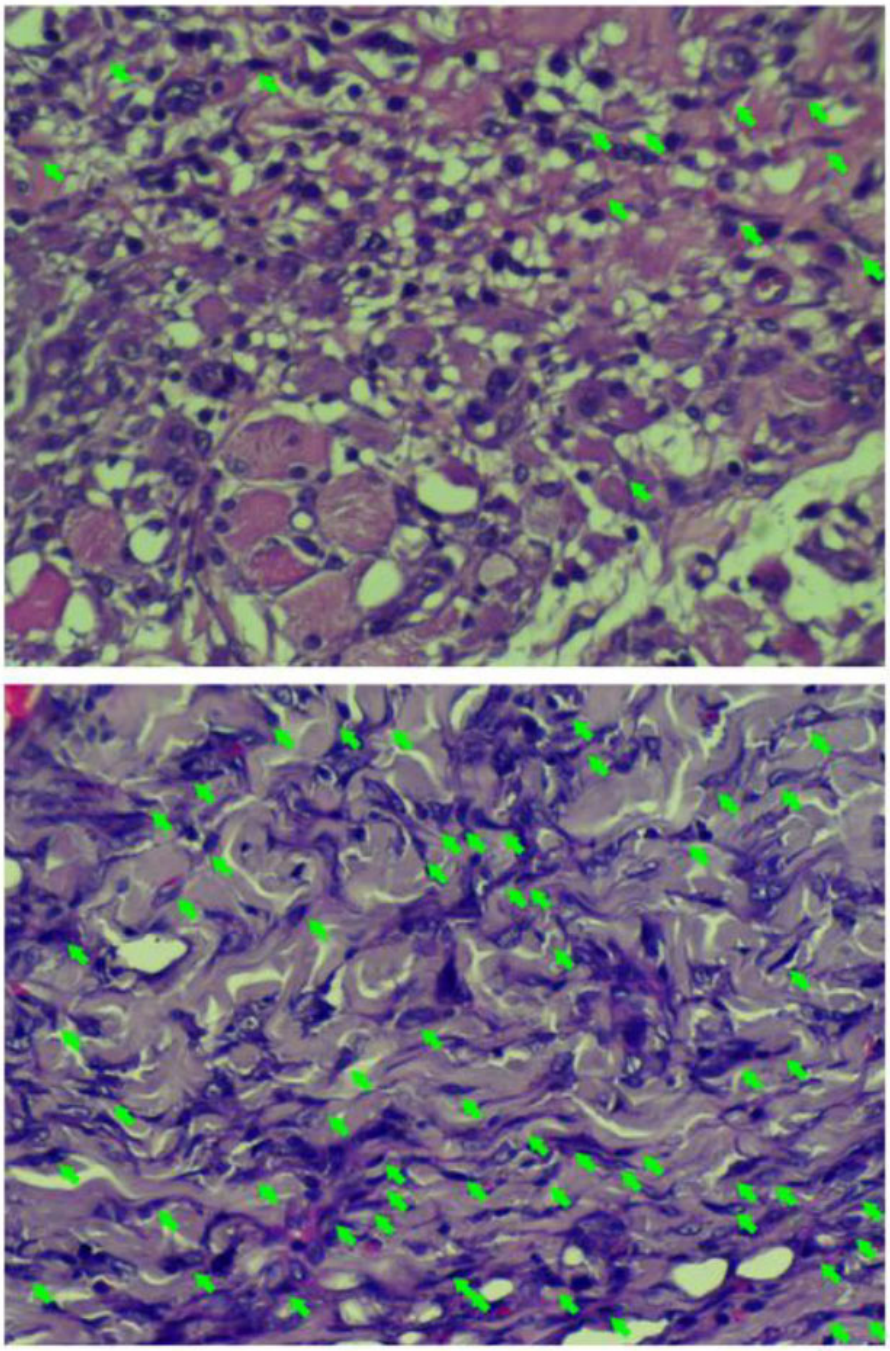

Figure 2. Fibroblasts (green arrows) observed in histological preparations of group K2 (top) and P2 (bottom) stained with Hematoxyline-Eosin (HE); 400x magnification.

is positive and above the range of 0.60, indicating a very strong correlation. The higher the speed of epithelialization, the higher the number of fibroblasts, respectively, in the control and treatment groups (r = 0.638; p = 0.014 vs. r= 0.608; p = 0.021). Similar values also occurred in the epithelialization relationship, which increased with the percentage of collagen tissue area in the control and treatment groups (r = 0.705; p= 0.005 vs. r= 0.784; p = 0.001). The increase in the number of fibroblast cells was followed by the rise in the percentage of collagen tissue area in the burn scar area of II A - degree, respectively, in the control and treatment groups (r = 0.754; p = 0.002 vs. r = 0.591; p = 0.026).

DISCUSSION

The results of this study indicated that topical administration of curcumin gel accelerated the healing process in second-degree burns of Wistar rats compared to paraffin gauze. Histopathological examination was performed to assess wound healing microscopically by counting the number of fibroblasts and the thickness of burn wound collagen on days 7 and 14 between the control and treatment groups. Macroscopically, a clinical assessment of epithelialization was carried out using digital imaging.

Using 1% topical curcumin gel in Wistar rat burns is considered the main role of curcumin as an anti-inflammatory. The previous research used a 2% concentration of curcumin gel on periodontitis in Wistar rats and proved effective as a good anti-inflammatory for 24-48 hours by reducing gingival inflammation.[7] The toxicity of doses exceeding 2% has not been proven clinically. Even though the inflammatory response in burns is higher than in other wounds, inflammation is still needed in injured tissue to stimulate cells to differentiate according to the needs of burn healing. Therefore, lower concentrations are considered in this study, with the hope that there will be a balance between inflammatory and anti-inflammatory reactions in burns.

Curcumin Gel on Number of Fibroblasts

Wound healing is a complex and dynamic process involving cellular and molecular events. The process is divided simply into three phases: (1) hemostasis and inflammation, (2) proliferation with the formation of granulation tissue, and (3) remodeling with the formation of new epithelium and scar tissue. In line with this process, the current study resulted in a significant study of fibroblast growth in the burn area between the curcumin-treated and non-curcumin-treated groups. The group that was given curcumin showed progress in increasing the number of fibroblast cells extensively and significantly accelerated the healing of the burn area.

During the inflammatory phase, many neutrophils migrate to the wound site, releasing proteases, ROS,

and inflammatory mediators such as TNF-α and IL-1. Curcumin can reduce inflammation through inhibition of NF-κB and suppression of TNF-α expression, as well as through interference with LPS signaling. In addition, curcumin exerts its anti-inflammatory effects by acting on other signaling pathways, such as peroxisome proliferator-activated receptor-gamma (PPAR-γ) and myeloid differentiation co-receptor protein 2-TLR4 (TLR4-MD2). Excessive oxidative stress plays a major role in prolonged inflammation. Low ROS levels are formed physiologically during the physiological process of wound healing. At the same time, their excessive production cannot be counterbalanced by the cellular antioxidant system, which causes oxidative stress, lipid peroxidation (LPx), DNA damage and enzyme inactivation. Curcumin restores redox balance and suppresses oxidation-related transcription factors while maintaining the production and activity of antioxidant enzymes and their constituents, such as glutathione (GSH). In addition, the protective action of curcumin against hydrogen peroxide has been observed in vitro in human keratinocytes and fibroblasts.[9]

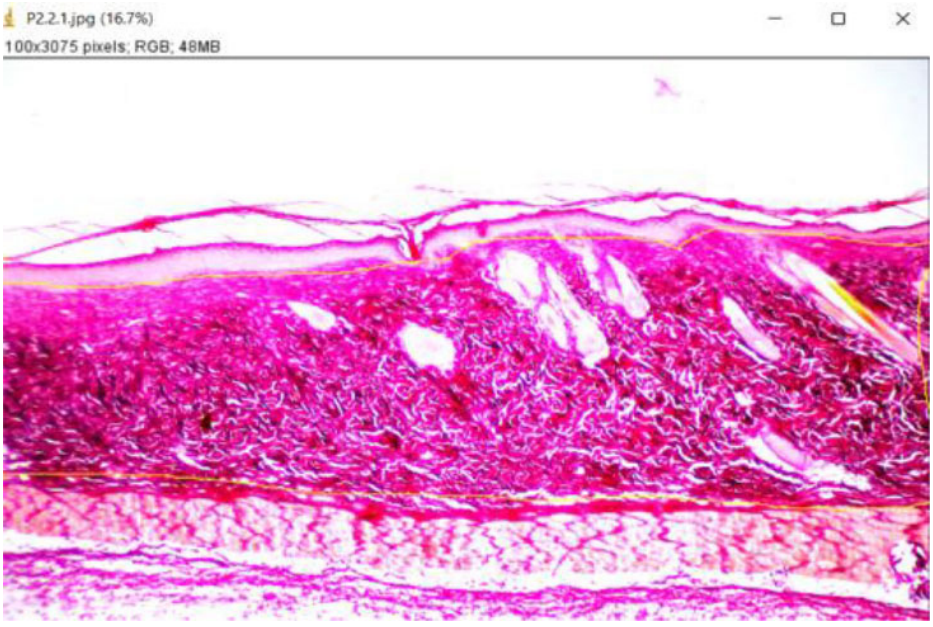

Figure 3. Collagen density (thin yellow line) observed in histological preparations stained with picrosirius red; 400x magnification.

Table 5. Summary of independent t-test of epithelialization rate

Variable	Groups	Mean ± SD	*Mean diff.*	t	p
Early epithelialization	K2	57.31 ± 8.95	4.67	-1.07	0.30
	P2	61.97 ± 7.18			
Late epithelialization	K2	75.82 ± 9.57	9.16	-2.09	0.05*
	P2	84.97 ± 6.52			
Overall Epithelialization	Early day	60.46 ± 6.80	12.01	-5.07	0.00*
	Pre-biopsy	72.46 ± 10.54			

Description: *Significancy (p<0.05)

Table 6. Independent t-test for the number of fibroblasts

Variable	Groups	*Mean* ± SD	*Mean diff.*	t	p
Number of Fibroblasts on day-7	K1	8.87 ± 4.49	14.94	-3.70	0.003*
	P1	23.80 ± 9.68			
Number of Fibroblasts on day-14	K2	26.47 ± 9.32	10.77	-2.16	0.05*
	P2	37.25 ± 9.31			
Total Fibroblast Count	Control	17.67 ± 11.53	12.85	-2.96	0.007*
	Intervention	30.52 ± 11.48			

Description: *Significancy (p<0.05)

Table 7. Independent t-test of collagen density in the treatment and control groups

Variable	Groups	*Mean* ± SD	*Mean diff.*	t	p
7th-day collagen density	K1	9.88 ± 6.32	21.66	-7.56	0.000*
	P1	31.54 ± 4.18			
14th-day collagen density	K2	49.06 ± 6.30	22.36	-7.53	0.000*
	P2	71.42 ± 4.69			
Total collagen density	Control	29.47 ± 21.22	22.00	-2.75	0.011*
	Intervention	51.48 ± 21.13			

Description: *Significancy (p<0.05)

Table 8. Correlation matrix between study groups on parameters of epithelialization, fibroblast count, and collagen density

Groups	Intercorrelation between Variable		Fibroblast Count	Collagen Density
Control	Epithelialization	r	0.638	0.705
		P-value	0.014*	0.005*
	Collagen Density	r	0.754	
		P-value	0.002*	
Intervention	Epithelialization	r	0.608	0.784
		P-value	0.021*	0.001*
	Collagen Density	r	0.591	
		P-value	0.026*	

Description: *Significance of Pearson correlation (p<0.05)

A previous study using *Rattus novergicus* in four groups (the wound group treated with carbomer, 0.9% NaCl, tulle and topical curcumin extract) compared the number of fibroblasts, in which there was a significant difference in the number of fibroblasts between the four experimental groups (p = 0.034). There was a considerable difference in the number of fibroblasts in the curcumin extract topical group compared to the tulle group.[10]

Treatment with curcumin is less toxic in some normal cells, such as mouse hepatocytes, mammary epithelial cells, human gingival fibroblasts, human lung epithelial cells, and prostate epithelial cells. At concentrations higher than 10 μM, curcumin would induce cell death in normal dermal fibroblast cells. The different findings from this research may be due to the differences in the tissue of origin of the fibroblasts studied. That study also added that epigenetic modifications in the promoter region express several fibroblast genes differently. This regulation is dependent on external stimuli (such as stress), tissue origin, or programmed gene activation temporarily after curcumin administration. The cytotoxic effect observed after curcumin treatment may be due to post-translational modifications (PTM) of histone molecules, which lead to modulation of gene expression. In this study, curcumin was given to skin areas induced by second-degree burns, where the fibroblast cell content in the dermal layer was quite responsive to curcumin administration.[11]

Curcumin Gel on Collagen Density

Apart from micronutrients, other compounds such as arginine and glutamine, vitamins A, B, C, and D, zinc, and iron are important for inflammatory processes and collagen synthesis. In particular, curcumin's anti-inflammatory and antioxidant properties can reduce the expression of tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) and restore the imbalance between ROS production and antioxidant activity. Since curcumin induces apoptosis of inflammatory cells during the initial phase of wound healing, it can also accelerate the healing process by shortening the inflammatory phase. In addition, curcumin can facilitate collagen synthesis, fibroblast migration, and cell differentiation. Although curcumin can be considered a wound healing agent, especially when administered topically, the injured patient is encouraged to adopt an appropriate nutritional approach to wound management.[12]

Concerning burn wound healing, past research has shown that collagen fibers mature earlier when wounds in mice are treated with curcumin. Although curcumin does not appear to be involved in the migration of fibroblasts into the wound area in vitro, an in vivo study suggests that curcumin mediates the infiltration of fibroblasts into the wound, which in turn naturally differentiate into myofibroblasts during granulation tissue formation.[13] This finding may be due to the difficulty in modeling the *in vitro* migration of fibroblasts in wounds. Treatment with curcumin also encourages the differentiation of fibroblasts into myofibroblasts, which marks the start of wound contraction until the formation of collagen expands in the dermis tissue.

In addition, from a molecular pathway, treatment with curcumin-loaded polymers resulted in significantly lower expression of PI3K and pAKT, indicating inhibition of the PI3K/AKT/NFκB axis, decreased levels of LPx, and increased considerably collagen compared to control.[14] However, contrary to these findings and the current research, the experimental research in 2020 revealed that there was no significant difference in collagen density between the wound groups treated with topical curcumin and tulle extracts.[10]

Curcumin reduces inflammatory conditions involving collagen formation through the "intestinal-brain axis" by modulating the function of the cholinergic system. A surprising study in 2018 showed histopathological changes after injecting bovine collagen type II and administering curcumin and methotrexate orally daily (hematoxylin-eosin staining).[15] Cytokine levels (TNF-α, IL-1β, IL-17 and TGF-β) were detected by radioimmunoassay, while IκBα and COX-2 expressions were detected by Western blot. In addition, cell viability was detected by the CCK-8 assay, and the effect of curcumin on macrophage apoptosis was detected by flow cytometry and TUNEL assay. The results showed that in vivo curcumin reduced cytokine levels, inhibited IκBα degradation, reduced COX-2 production in LPS-induced inflammatory cells, and significantly induced macrophage apoptosis.

Curcumin also significantly affects the thermal properties of collagen. The dynamic surface tension remained nearly constant in collagen without curcumin, whereas for curcumin-treated collagen, surface tension decreased with time by a ratio of 1:100 each. This suggests that although higher concentrations of

curcumin increased the surface activity of collagen, this activity decreased over time. Curcumin administration can also cause changes in the local restructuring of water molecules, which cause exposure to non-polar groups on the surface area of collagen, thereby increasing the surface activity of collagen. Over time, it will decrease again due to reduced water molecules.[15] This concept is well studied when wound healing is in progress from the beginning until wound closure occurs, which saves the body's microcellular biochemistry in wound healing.

Curcumin Gel on Epithelialization Rate

Recent literature on wound healing properties also provides evidence of curcumin's ability to enhance granulation tissue formation, collagen deposition, tissue remodeling and wound contraction. Optimizing the application of topical curcumin through its formulation is very important to ensure the maximum therapeutic effect of curcumin on burns.[16] These findings align with a follow-up study that suggested that curcumin nanogels could potentially increase the penetration of curcumin in the skin, and dermal localization protects it from the effects of degradation.[17]

An experimental study was designed and conducted at the Post Graduate Medical Institute, Lahore, to evaluate the role of curcumin in post-inflammatory epithelialization and collagenization of the buccal area of guinea pigs. Forty adult rabbits were divided into 20 samples in group A and 20 in group B. Each group was further divided into two groups, 10 each as the treatment on the 5th and 7th day after burns occurred on the buccal mucosa. Group A animals were left without treatment while group B animals were given curcumin 30 mg/kg body weight daily orally gavage. Histological analysis revealed that animals treated with curcumin experienced faster epithelialization and collagenization than the control group, thus indicating the effectiveness of curcumin as an anti-inflammatory agent in an inflammatory process.[18]

Moreover, a study in 2015 showed curcumin as a cheaply available herb proved to be a suitable substitute for healing burns.[5] Their study used seventy female Sprague-Dawley rats weighing 180-220 grams randomly divided into 5 equal groups. Administration of curcumin resulted in a decrease in burn size and a reduction of inflammation after 14 days.

The curcumin treatment increased the expression of caveolin-1 in epidermal stem cells, which is required as an opportunistic effect of curcumin on epidermal cell proliferation. Their study using experimental mice showed that epidermal cells treated with curcumin experienced increased wound closure with the help of caveolin-1 expression.[19] During the proliferative phase of wound healing, the dermis is invaded by proliferating fibroblasts that produce immature ECM proteins (EDA fibronectin and type III collagen) and activate growth factors such as TGF-β1, which promote repair of injured dermal layers. Simultaneously, keratinocytes migrate at the injured site, where they proliferate vegetatively and differentiate to restore the overlying epithelium. The main role in this process is played by hair follicle stem cells. Curcumin can stimulate significant actions during the proliferative phase. It has been demonstrated that curcumin can reduce the number of membrane matrix metalloproteinases (MMPs), increase hydroxyproline and collagen synthesis, and accelerate collagen fibers' maturation. In addition, curcumin also promotes the differentiation of fibroblasts into myofibroblasts, which marks the start of wound contraction and reduces the period of epithelialization in wounds.[9]

LIMITATIONS

In the future, it is hoped that curcumin gel can be used as a therapy for treating burns in humans if it has been clinically tested. The limitation of this study is that it did not examine variables to determine how curcumin works on inflammatory mediators, genetic studies in the growth of fibroblasts and collagen cells, and their toxicity. Due to time constraints in this study, no observations were made on the wound during the remodeling phase, so the quality of the resulting scars is unknown.

CONCLUSION

Wound treatment with topical curcumin gel accelerated epithelialization, increased the number of fibroblasts, and increased collagen thickness in male Wistar rats (*Rattus norvegicus*) burns compared to Paraffin Gauze.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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ETHICAL CLEARANCE

The Health Research Ethics Committee of the Faculty of Medicine, Udayana University No. 2755/UN14.2.2 VII.14/LT/2022 provided this study's ethical clearance.

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