

Effectiveness of topical turmeric extract compared to topical zinc on fibroblast count and collagen density in clean-contaminated wound: a study on new zealand white rabbit



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ABSTRACT

Introduction: Wound dehiscence increased the average mortality and morbidity, patient care cost and health services. Therefore, a more effective and lower-cost therapy is needed for wound healing. Zinc is a trace element which is important in the wound-healing process, while curcumin is an active component of turmeric which can also help the wound-healing process. This study aims to compare the effectiveness of topical turmeric extract with topical zinc on fibroblast count and collagen density in clean-contaminated wounds in New Zealand white rabbits.

Material and Methods: This study was a randomized experimental study in 30 New Zealand white rabbits with clean contaminated wounds divided into 3 groups. One control group (treated with NaCl 0.9%) and two treatment groups (treated with topical turmeric extract and with topical zinc). Appendectomy was performed to all samples and surgical wounds were treated until day 4 postoperative. On the fifth day, histopathology observation at the edge of the surgical wound was done to see the fibroblast count using Haematoxylin Eosin staining and collagen density using Masson Trichrome staining.

Results: There was a significant difference in the number of fibroblasts between the control group (NaCl 0.9%), the topical zinc group and the topical turmeric extract group (Kruskal-Wallis $p=0.000$). The collagen density score also had significant differences in the three groups (Kruskal-Wallis $p=0.003$). Although the Mann-Whitney test in the topical zinc group and the topical turmeric extract group showed no significant difference in the collagen density score ($p=0.074$).

Conclusion: Topical administration of turmeric extract has been shown to affect the number of fibroblasts and collagen density in clean-contaminated wounds in New Zealand white rabbits, as well as topical zinc administration.

Keywords: topical zinc, topical turmeric extract, clean contaminated wound.

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INTRODUCTION

Wound healing is a dynamic process consisting of three main phases, they are the inflammatory phase, the proliferative phase, and the maturation phase. The proliferative phase is closely related to the inflammatory response and plays an important role in the final inflammation process.¹ Infection can occur when microorganisms in a wound proliferate to a levels that can produce local and/or systemic response. Infection increases the production of degradative enzymes by immune cells and bacteria which can interfere with healing process and weaken wound tissue.² As a result, infection of the operating area can occur and lead to wound dehiscence.³ Abdominal surgical wound dehiscence is a severe postoperative

complication with a reported mortality rate of 45%, in emergency surgery the incidence rate is 16.67% higher than elective surgery.⁴ A multicenter study in the Netherlands showed 42 of 637 patients had surgical wound infections after appendectomy, including acute appendicitis 64.7%, perforated appendicitis 17.3%, gangrenous appendicitis 11.3%, and purulent appendicitis 23.2%.⁵ In addition to increasing the average mortality and morbidity, wound dehiscence also increases patient care costs and health services.⁶ Although there are various therapeutic modalities for wound healing, they are not effective enough. Therefore, a more effective therapy is needed for wound healing and also a lower cost.⁷

Zinc is a trace element that is

important for various aspects of health.⁸ Zinc deficiency in the body can cause various manifestations, including failure in the wound-healing process.⁹ At the meeting of the World Union of Wound Healing Societies in Paris in 2004 it was stated that zinc has good abilities in the wound healing process.¹⁰ Herbal medicine traditionally has been widely used and the plant parts used are not only taken from the plant part, but can come from other, more complex forms such as extracts or in the form of mixtures with other ingredients.^{11,12} Curcumin is an active component of turmeric that contains antioxidants, anti-inflammatory, and anti-carcinogenic which has long been used as a medicine for various diseases in Southeast Asian countries. Various studies

have stated that curcumin helps the wound healing process even in difficult conditions such as radiation injuries, burns, laser injuries, or diabetic wounds.

MATERIAL AND METHODS

This is an experimental study using randomization techniques in taking rabbit samples. A total of 30 experimental animals were divided into three groups: one control group (clean contaminated wounds treated only with 0.9% NaCl) and two treatment groups (contaminated clean wounds treated with topical turmeric extract and topical zinc). Before receiving treatment, the rabbits in this study also underwent a physical examination and adaptation process for 7x24 hours to ensure that the rabbits did not suffer from a disease or had no potential to transmit disease. The inclusion criteria in this study were healthy and active New Zealand White rabbits (*Oryctolagus Cuniculus*), male, aged 6 - 9 months, with body weight 2 - 3 kilograms. The exclusion criteria in this study were rabbits that during the adaptation period behaved aggressively and showed illness. The independent variables in this study were topical turmeric extract and topical zinc used in clean contaminated postoperative wounds with the dependent variable being collagen deposition and the number of fibroblasts in the abdominal wall laparotomy incision.

All samples underwent the procedure for making clean-contaminated wounds, by performing a laparotomy appendectomy and then treating the surgical wound until day 4 postoperative (Figure 1). On the 5th day postoperative, histopathological observations were conducted on the edge of the surgical wound to see the number of fibroblasts using Hematoxylin Eosin staining and to see the collagen density based on the collagen score using Masson Trichrome staining. There are 5 collagen density scores, they are score 0 which indicating no collagen fiber in the wound area, score 1 indicating low collagen fiber density in the wound area (10-25%), score 2 showing moderate collagen fiber density in the wound area (25-50%), score 3 indicates dense collagen fiber density in the wound area (50-75%), and score 4 indicates very

dense collagen fiber density in the wound area (75-100%).¹³ The hypothesis test used to determine differences in the number of fibroblasts in the three groups was the one-way ANOVA test (if the data distribution is normal) or the Kruskal-Wallis test (if the data distribution is not normal), while the Kruskal-Wallis test was used to test the comparison of collagen density variables.

RESULTS

A non-parametric test (the Kruskal-Wallis test) was used to analyze the number of fibroblasts in the three groups because the data was not normally distributed. Based on the results of the Kruskal-Wallis test, there were significant differences for the number of fibroblasts between the control group (NaCl 0.9%), the topical zinc treatment group, and the topical turmeric extract treatment group, with p-value <0.001 ($p < 0.05$), fibroblast histology comparison between three groups can be seen in figure 2. The Mann-Whitney test was carried out to find out which groups are different. Based on the results of the analysis of the Mann-Whitney test in the control group (0.9% NaCl) and the topical zinc treatment group, the results showed a significant difference of the number of fibroblasts with p-value <0.001 ($p < 0.05$). In the control group (0.9% NaCl) and the topical turmeric extract treatment group, the test also showed a significant difference of the number of fibroblasts with

p-value <0.001 ($p < 0.05$). For the topical zinc treatment group and the topical turmeric extract treatment group, the Mann-Whitney test resulted a significant difference of the number of fibroblasts with p-value 0.006 ($p < 0.05$) (Table 1).

Based on the Kruskal-Wallis test for collagen density scores between the control group (NaCl 0.9%), the topical zinc treatment group, and the topical turmeric extract treatment group, it is known that the collagen density score was significantly different between the three groups with p-value 0.003 ($p < 0.05$), collagen density histology comparison between three groups can be seen in figure 3. Similar to the analysis test for the number of fibroblasts above, the Mann-Whitney test was conducted to determine which groups differed. Based on the Mann-Whitney test analysis in the control group (0.9% NaCl) and the topical zinc treatment group, the collagen density score was significantly different with p-value 0.035 ($p < 0.05$). In the control group (0.9% NaCl) and the topical turmeric extract treatment group, the Mann-Whitney test also showed a significant difference of collagen density score with p-value 0.002 ($p < 0.05$). In contrast to the Mann-Whitney test in the topical zinc treatment group and the topical turmeric extract treatment group, the collagen density score between these two groups was not significantly different with p-value 0.074 ($p > 0.05$). (Table 2)

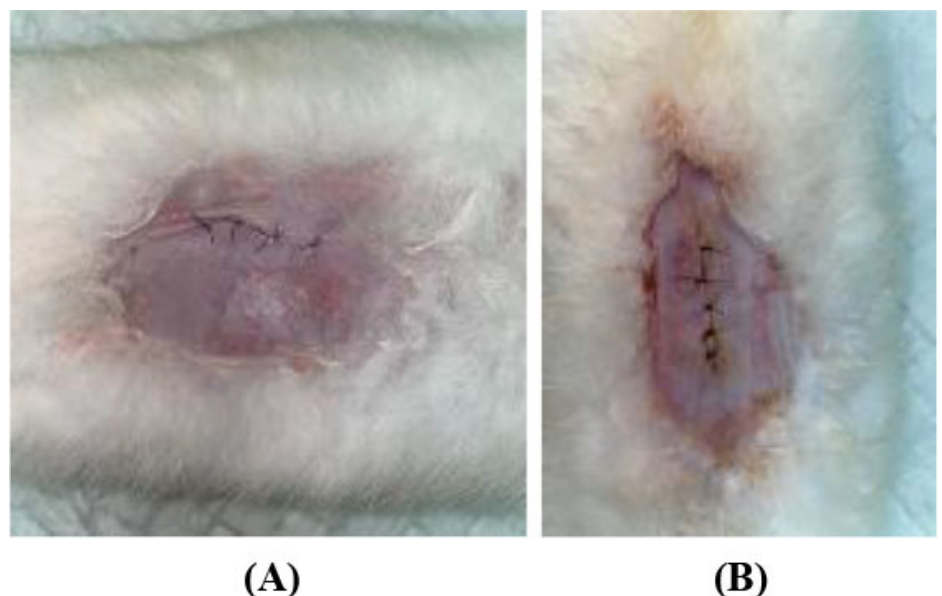


Figure 1. (A) Wound is treated with topical Zinc, (B) Wound is treated with topical turmeric extract.

DISCUSSION

In this study, there was a significant difference in the number of fibroblasts between the control group (NaCl 0.9%), the topical zinc treatment group, and the topical turmeric extract treatment group. In addition, the highest number of fibroblasts was found in the topical turmeric extract treatment group, then in the topical zinc treatment group, and the lowest in the

control group (0.9% NaCl). The above results are supported by previous studies which stated that there were significant differences in the number of fibroblasts and collagen in wound healing between the administration of topical zinc and topical turmeric extract. Research by Magnus and colleagues in 1990 stated that topical zinc administration increased epithelialization of partial thickness wounds in pigs at 48 and 64 hours post-injury.^{14,15}

Topical application of zinc which causes the closure of the entire epidermal layer and part of the dermis on the fifth day after the incision wound is related to the function of zinc in accelerating re-epithelialization, fibroblast proliferation, and collagen synthesis.¹⁶ Experimental models of wound healing in rats demonstrated that within 24 hours of injury, there was a 15% to 20% increase in zinc levels at the wound edges, and this increased by up to 30% during granulation tissue growth and subsequent epidermal proliferation.¹⁶ The role of zinc in this proliferative phase is that zinc acts as a cofactor for TGF- β and SMAD which then triggers the appearance of fibroblasts and collagen in the wound tissue, this process lasts until the deposit of fibroblasts and collagen is considered sufficient to become the foundation structure for cell migration and the re-epithelialization process.¹⁷

Curcumin is the most abundant active form among the three forms of curcuminoid.¹⁸ Mohanty et al. in 2012 conducted a study on rats that were given curcumin topically on excision wounds and showed that the formation of fibroblasts went better than wounds that were only treated using ordinary gauze and primary suturing.¹⁹ Curcumin induces apoptosis of inflammatory cells

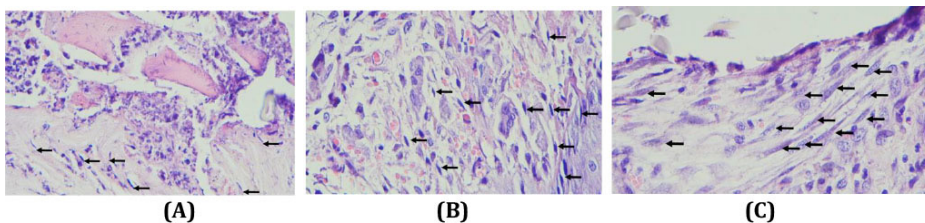


Figure 2. The number of fibroblasts using Hematoxylin Eosin staining: (A) the control group (NaCl 0.9%), (B) the topical zinc treatment group, (C) the topical turmeric extract treatment group.

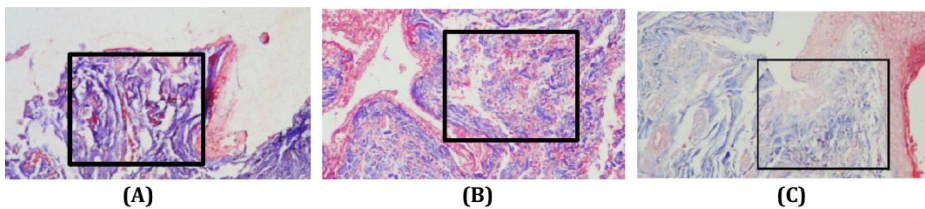


Figure 3. The collagen density using Masson Trichrome staining: (A) the control group (NaCl 0.9%), (B) the topical zinc treatment group, (C) the topical turmeric extract treatment group.

Table 1. Analysis of the number of fibroblasts between the control group (NaCl 0.9%), the topical zinc treatment group, and the topical turmeric extract treatment group.

	N	Range (median)	Mean \pm SD	p-value (Kruskal-Wallis)	p-value NaCl : topical zinc (Mann-Whitney)	p-value NaCl : topical turmeric extract (Mann-Whitney)	p-value topical zinc : topical turmeric extract (Mann-Whitney)
NaCl	10	2-6 (3)	3.3 \pm 1.16	0.000		0.000	
Topical zinc	10	6-12 (8)	8.5 \pm 1.90		0.000		
Topical turmeric extract	10	8-13 (11.5)	11.2 \pm 1.68				0.006

Table 2. Analysis of collagen density scores between the control group (NaCl 0.9%), the topical zinc treatment group, and the topical turmeric extract treatment group.

	p-value (Kruskal-Wallis)	p-value NaCl : Topical zinc (Mann-Whitney)	p-value NaCl : Topical turmeric extract (Mann-Whitney)	p-value Topical zinc : Topical turmeric extract (Mann-Whitney)
NaCl	0.003		0.002	
Topical zinc		0.035		
Topical turmeric extract				0.074

in the early phase of wound healing, inhibits the activation of the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), reduces the production of inflammatory cytokines such as tumor necrosis factor alpha (TNF- α) and interleukin 1 (IL-1), removes reactive oxygen species (ROS), affects the production of antioxidant enzymes, to reduce the inflammatory reaction and shortening the duration of the inflammatory phase with the result of accelerating the wound healing process to enter the next healing phase, namely the proliferative phase with the formation of fibroblasts.^{19,20}

The curcumin's complex action can affect the proliferative stage of the wound as described above, so this might be the basis of our research results which were the highest number of fibroblasts result were found in the topical turmeric extract treatment group, then in the topical zinc treatment group, and the lowest in control group (NaCl 0.9%). This study also found significant differences in collagen scores between the control group (NaCl 0.9%), the topical zinc treatment group, and the topical turmeric extract treatment group. In addition, there were significant differences in collagen scores between the control group (0.9% NaCl) and the topical zinc treatment group, the control group (0.9% NaCl) and the topical turmeric extract treatment group. Still, between the topical zinc treatment group and the topical turmeric extract treatment group the collagen score was not significantly different even though the collagen score 3 and collagen score 4 were most commonly found in the study sample of the treatment group that was given topical turmeric extract compared to the treatment group that was given topical zinc.

On the third day of wound healing, fibroblasts begin synthesizing and secrete some collagen, which will continue increasing for about three weeks.²¹ In vitro studies, it was stated that zinc helps the ECM-remodeling matrix metalloproteinases (MMPs) to function optimally. MMPs are a protein that plays a role in the epidermal wound healing process. The coordinated function of MMPs is critical in the migration of

keratinocytes, neoangiogenesis, cell proliferation, activation/migration of fibroblasts, and deposition of collagen and formation of fibrous tissue.^{17,22}

Research conducted by Panchatcharam and colleagues in 2006 in India stated that topical curcumin therapy (given once every 24 hours for 8 days) on full-thickness wounds in the skin of white rats significantly increased collagen density on the fourth to eighth day.²³ In a study with rats, collagen levels in wounds treated with CLCA were higher than in the control group treated with sterile gauze. The collagen produced was denser and neatly arranged and thicker in the group that received curcumin therapy. Compared with oral administration, it was found that topical administration of curcumin resulted in the formation of denser and more organized collagen in rats with diabetes.²⁴ We did not find studies comparing topical zinc and topical turmeric extract in wound healing.

The results of the collagen score between the topical zinc treatment group and the topical turmeric extract treatment group were not significantly different, this could be due to the data used in this study being ordinal data, so further research may be carried out using data samples with various categories from this study and a larger number of pieces. It is expected that further research not only uses scores that indicate the increase in collagen in wound healing but also examines the types of collagen that are affected.

Although the results of the collagen score between the topical zinc treatment group and the topical turmeric extract treatment group were not significantly different, topical turmeric extract and topical zinc had significant differences of collagen score compared to the control group (NaCl 0.9%). In other words, in clean contaminated wound healing topical turmeric extract is as good as topical zinc and both are better when compared to treatment using only 0.9% NaCl. The basic ingredient of turmeric which is easy to obtain and cheap are the advantages of topical turmeric extract compared to topical zinc. However, until now there is no topical turmeric extract preparation on the market.

CONCLUSION

Topical administration of turmeric extract and zinc has been shown to affect the number of fibroblasts and collagen density in clean-contaminated wounds in New Zealand white rabbits.

RESEARCH ETHICS

This research has been approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Airlangga, with ethic number 2.KEH.086.07.2022, and has carefully studied the proposed animal use protocol.

AUTHOR CONTRIBUTION

All authors have made the same contribution in writing the report on the results of this study, from the stage of proposal preparation, data search, and data analysis to the interpretation of research data and presentation of the final report.

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DISCLOSURE OF INTEREST

The author reports no conflicts of interest in this work.

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