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The Effects of Turmeric Extract (*Curcuma longa*) Loaded Hidrogels in Accelerating Wound Closure

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Abstract

Background: Disruption to healing process results on longer healing duration from the timeline. Povidone iodine and tulle are common management in wound care. The advance of industry in health sector and the availability of natural ingredients in Indonesia provide opportunities for developing alternatives for wound care. Turmeric (*Curcuma longa*) contains curcumin which is anti-inflammatory, antibacterial, and antioxidant; it is good for wound healing. Hydrogel nanocomplexes of carboxymethyl chitosan can maintain moisture in wound area and protect curcumin from hydrolysis. **Objective:** To analyze the potential of turmeric extract loaded hidrogels in accelerating wound closure. **Method:** experimental research with "post-test only control group design". The sample consisted of 30 mice which were assigned into 5 groups. **Result:** All experimental groups experienced a gradual decrease in wound length. The statistical test results for groups II and III showed significant advantages compared to group IV ($p < 0.05$). Meanwhile, group I did not have significant differences in wound closure compared to group IV on day 1 and 3 ($p > 0.05$), but it had significant results ($p < 0.05$) on day 5. **Conclusion:** Turmeric extract loaded hidrogels can accelerate the reduction of wound length.

Keywords: Carboxymethyl Chitosan, Curcumin, Hydrogels, Turmeric, Wound Healing

Introduction

Wounds are damage to normal structures of the skin, either closed or open ones. Based on healing duration, wounds are classified into acute and chronic wounds¹. Chronic wounds occur due to disrupted wound healing process and it takes longer to heal than the timeline². There are more than 5.7 million chronic wound patients in the US and it is estimated that the annual medical costs reaches US \$20 billion. The cost is expected to increase sharply to US \$24.8 billion by 2024³. Therefore, proper wound healing is important in order to reduce costs and prevent chronic wounds⁴.

Wound healing process consists of several phases; coagulation and hemostasis phase, inflammatory phase, proliferation phase, and remodeling phase^{5,6}. Povidone iodine is a common management in wound care. The advance of industry in health sector and the availability of natural ingredients in Indonesia provide opportunities for developing better alternatives for wound care.

Curcumin from turmeric extract (*Curcuma longa*) is a polyphenol that has anti-inflammatory, antibacterial, and antioxidant properties^{7,8}. In addition, it increases granulation tissue formation, epithelial regeneration, and angiogenesis when topically administered to wounds⁹. However, curcumin has hydrophobic property which hinders its topical administration directly on wounds^{3,10}. Carboxymethyl chitosan is a chitosan derivative that is non-toxic, biocompatible, and water-soluble^{11,12}. In the process of wound healing, carboxymethyl chitosan can

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stimulate cell proliferation, increase collagen formation, as well as accelerate regeneration and epithelialization of wound tissues¹³.

The advance on health industry has provides alternatives for wound care modalities, one of which is the use of biopolymers¹⁴. Hydrogel is a cross-polymer network that is hydrophilic. Therefore, it is suitable for wound healing process because it can maintain moisture in wound area, ensure air exchange for tissue respiration, protect curcumin from hydrolysis, and provide comfortable sensation when used¹⁵. This research investigated the potential use turmeric extract loaded hidrogels and answered the problem of the efficiency turmeric extract loaded hidrogels to accelerate wound healing process by indicators of wound length.

Materials and Methods

Turmeric Extraction:

Turmeric (*Curcuma longa*) were washed, drained, and weighed. Turmeric were cut into thin strips and air-dried in the shade. To maximize the drying process, turmeric slices were roasted at 45°C for about 48 hours or until completely dried. The dried turmeric slices were crushed in a blender and sieved into powder. Turmeric powder then extracted with 96% ethanol using maceration method. Extraction process was continued with rotary evaporator to removed the alcohol content and a thick extract will be produced. The extraction process was carried out by the UPT Laboratorium Herbal Materia Medica Batu, Indonesia.

Preparation of Hidrogels:

One gram of carbomer 940 was dispersed in 100mL of distilled water. One gram of carboxymethyl chitosan was dissolved in 100mL of distilled water. Carboxymethyl chitosan and carbomer 940 disperses were mixed at 400 rpm for 5 minutes using a magnetic stirrer. Then, triethanolamine was added to the carboxymethyl chitosan-carbomer 940 hydrogel until its pH reached 6 and was homogeneous. Turmeric extract was added with different volumes in each group; 1 mL for group I, 2 mL for group II, and 3 mL for group III. Each solutions was added with carboxymethyl chitosan-carbomer 940 hydrogel until it amounted 100mL.

Research Design:

This research has been confirmed the ethical conduct by the Health Research Ethics Committee of Medical Faculty of Airlangga University with the issuance of statement No.58/EC/KEPK/FKUA/2020. The location of this study was carried out in the laboratory of the Department of Pharmacology of the Faculty of Medicine, Airlangga University in February 2020. The design was experimental research through testing on experimental animals (in vivo study) with a “post-test only control group design” approach. The sample size was obtained from the Federer formula, $[(t-1)(n-1)] \geq 15$ where t is the number of treatments / number of groups and n is the number of repetitions / sample size in the groups¹⁶. The calculation gave result of sample size at 30. Mice (*Mus musculus*) were used and assigned into five groups. The following was the division of the research groups:

Group I : Mice were given 1% turmeric extract loaded hidrogels with a topical dose of 2 time 0.5 mL/day during treatments.

Group II : Mice were given 2% turmeric extract loaded hidrogels with a topical dose of 2 time 0.5 mL/day during treatments.

Group III : Mice were given 3% turmeric extract loaded hidrogels with a topical dose of 2 time 0.5 mL/day during treatments.

Group IV : Negative control, mice were given aquades with a topical dose of 2 time 0.5 mL / day during treatments.

Group V : Positive control, Mice were treated with tulle + povidone iodine with a topical dose 2 time 0.5 mL / day during treatments

Animal Model:

Adult male mice (*Mus musculus*), aged 8-12 weeks and weighed 20-30 grams, were used in this research and then acclimatized for 7 days. The hair on the back of the mice was shaved. Then, disinfection was carried out using 70% alcohol, followed by anesthesia which was carried out by intraperitoneal injection of ketamine 2 ml/kg/BW + 0.8 ml xylazine added to 10 ml of water and injected with a dose of 0.1 ml/kg/BW. The incisions were made 10 mm long and 2-3 mm deep in the back

of the mice. The data were in the form of reduction of wound length which were recorded on day 1, day 3, and day 5.

Statistical Analysis

Statistical analysis was performed using SPSS 22. The data presented as mean ± standard deviation (SD). After that, a two-way ANOVA statistical test was carried out followed by the Post Hoc LSD test to determine the effect of treatments. Significance was shown at $p \leq 0.05$.

Result

All groups in this research had good wound conditions and showed no sign of infection. On day 1, the wounds in all groups did not show any significant difference as they were still in inflammatory phase. On day 3, the wounds showed differences between groups I, II, and III, with group IV which was a negative control. In group I, there were granulation tissues but still looked red. In groups II and III, there were clear granulation

tissues. Meanwhile, in group IV, the wounds looked paler and more yellowish, indicating a lack of vascularization in wound area. On day 5, there were clear differences between wounds in groups I, II, and III, with group IV. The wounds in groups I, II, and III had re-epithelialized and almost completely closed, while in group IV the wound was wider. (Figure 1)

Wound length reductions were measured on day 1, day 3, and day 5 of treatments. The results can be seen in Table 1. Wound length reductions occurred gradually in all treatment groups. The results of mean wound length reduction on day 1 and day 3 showed that there were significant differences between groups II, III, and V compared to group IV as negative control ($p < 0.05$). Meanwhile, the mean wound length reduction in group I was not significant ($p > 0.05$). On day 5, the results of mean wound length reduction in groups I, II, III and V were significant to group IV ($p < 0.05$). The statistical test of wound length reduction which compared groups II and III did not show significant results ($p > 0.05$).

Table 1. Mean ± standart deviation (SD) of wound length

Day	Group I 1% turmeric extract loaded hidrogels (mm)	Group II 2% turmeric extract loaded hidrogels (mm)	Group III 3% turmeric extract loaded hidrogels (mm)	Group IV Aquades (mm)	Group V Tulle + Povidone iodine (mm)
1	8.0 ± 0.6	6.8 ± 0.7*	6.5 ± 1.0*	8.3 ± 0.5	7.3 ± 0.8*
3	6.1 ± 1.1	4.8 ± 1.1*	4.3 ± 1.2*	7.3 ± 0.8	5.0 ± 0.8*
5	4.6 ± 1.0*	3.1 ± 0.7*	3.0 ± 0.8*	6.0 ± 0.8	3.5 ± 1.0*

Figure 1. Wound closure for mice after 1, 3, 5 days of *in-vivo* incision wounds. (I) 1% turmeric extract loaded hidrogels (II) 2% turmeric extract loaded hidrogels (III) 3% turmeric extract loaded hidrogels (IV) aquades (negative control). (V) Tulle + Povidone iodine (positive control).

Discussion

This research attempted to analyze the potential of administering turmeric extract loaded hidrogels to reduce wound length in mice. From the results, it was found that turmeric extract loaded hidrogels could accelerate the process of reducing wound length.

In determining the concentration of turmeric extract with carboxymethyl chitosan nanocomplex hydrogel, this research considered that of *in vitro* study regarding the production of chitosan-based curcumin nanoemulsion gel where a concentration of 1% and 2% showed good spreadability and good gel quality, whereas a concentration of 4% showed poor spreadability¹⁷. Other research have shown that high curcumin concentration

increased the formation of ROS at the inflammatory stage that inhibit wound healing process¹⁸. These were used as the basis for determining the ratio of the active ingredient concentration 1; 2; 3 with the hope of producing quality hydrogel with optimal spreadability and minimizing material toxicity.

In group V, which was a positive control, povidone iodine covered with tulle was used. Tulle is a synthetic textile fiber which is a polyamide resin derivative which is elastic, anti-decay in nature, and contains lanolin to keep the wound moist with a semioclusive barrier to accelerate wound healing. Povidone iodine has antibacterial properties, so it can protect wounds from bacteria which can potentially prolong wound healing duration¹⁹.

In group III, which was given 3% turmeric extract loaded hidrogels, had the smallest wound length with a value of 3.0 ± 0.8 on day 5. Meanwhile, group IV as the negative control had the longest wound length with a value of 6.0 ± 0.8 on day 5, and was statistically significant compared to positive control group and the treatment groups I, II and III. Group III had a shorter wound length than group II on day 5 with a difference of 0.1 mm, and it was not statistically significant. These results indicate that administering turmeric extract loaded hidrogels can reduce wound length.

Interestingly, on days 1 and 3, there were no significant differences between group I who was given 1% turmeric extract loaded hidrogels and group IV which was a negative control. However, it showed significant results on day 5. The inflammatory phase is a natural response immediately after injury is modulated by pro-inflammatory cytokines. Based on previous research, it was reported that pro-inflammatory cytokines such as (IL) -1α , $IL-1\beta$, $IL-6$, $IL-12$, and $TNF-\alpha$ play a role in initiating inflammation, angiogenesis, leukocyte recruitment, and epithelialization in wound healing process. Therefore, the inflammatory phase is an important natural phase in wound healing process²⁰. However, an increased and prolonged expression of $TNF-\alpha$ and other pro-inflammatory cytokines can interfere with wound healing process²¹. The use of curcumin from turmeric extract can increase cells sensitivity to participate in the inflammatory response mediated by high $TNF-\alpha$ levels. After that, the $TNF-\alpha$

level will immediately return to the normal level to prevent prolonged $TNF-\alpha$ expression. In addition, it has been reported that curcumin can accelerate re-epithelization of wounds. This confirms that curcumin does not eliminate the inflammatory process itself, but prevents the prolonged pro-inflammatory cytokine activation which causes hypertrophic wounds and play a role in wound re-epithelialization. These results are consistent with research conducted by Niranjana et al. (2019) regarding the role of poly vinyl alcohol-chitosan-curcumin polymers in wound healing process in vitro and in vivo whose results showed that wound closure in the treatment group was better than the control group²².

Wound closure is the final stage of complex wound healing. This stage is influenced by previous phases; the inflammation and proliferation phase. In the process of reducing wound length, the results showed that the use of turmeric extract loaded hidrogels could accelerate healing because of the combined effect on each phase of wound healing by accelerating the inflammatory phase, helping in the proliferation phase, and playing a role by increasing MMP and inhibits TGF in remodeling phase^{7,23,24}.

Conclusion

This research proved that turmeric extract loaded hidrogels can accelerate the process of reducing wound length. The use of turmeric extract loaded hidrogels in group I did not show significant differences compared to the negative control group on day 1 and 3, but on day 5 there were significant results. Meanwhile, groups II and III showed significant advantages compared to the negative control group. Therefore, turmeric extract loaded hidrogels is a good modality in wound care management. However, it is necessary to carry out further analysis regarding the optimum topical dosage and the long-term effects of its administration.

Disclosure of Interest: The authors report no conflict of interest.

Ethical Clearance: This study had been approved by Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

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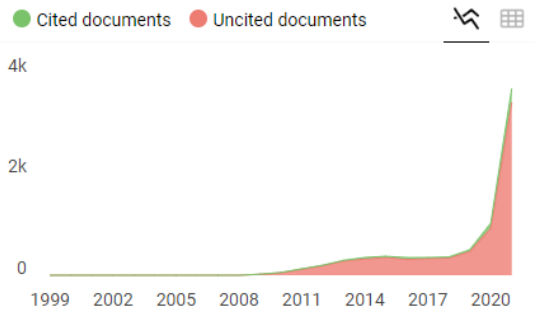
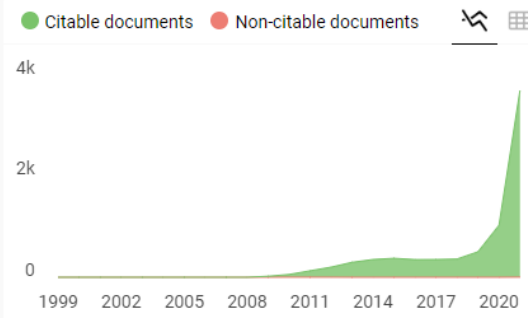
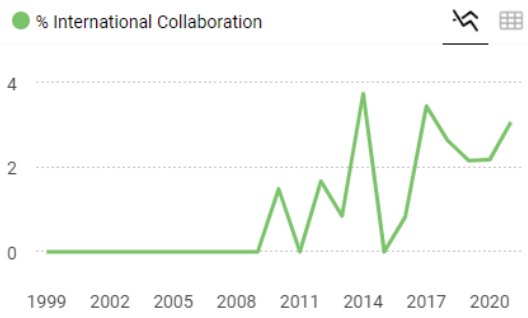
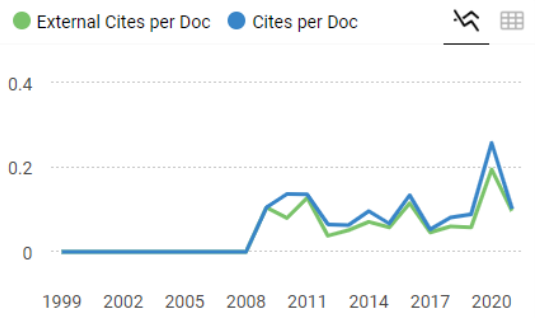
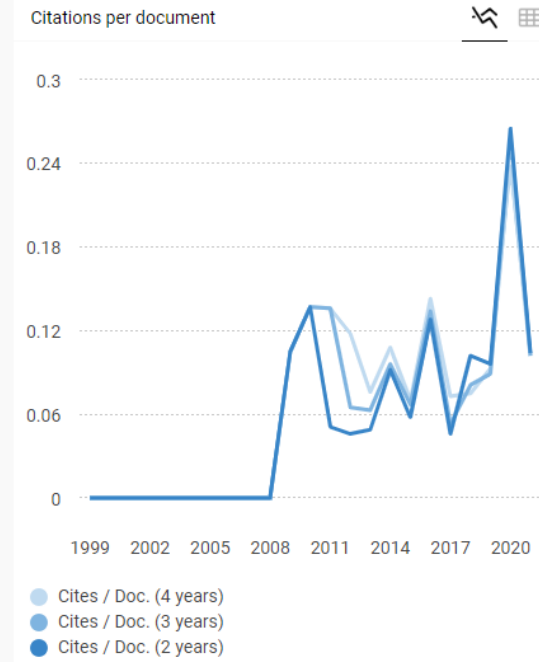
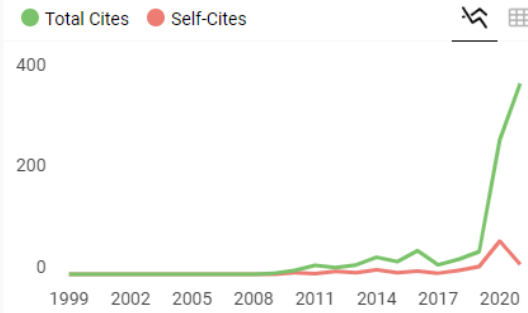
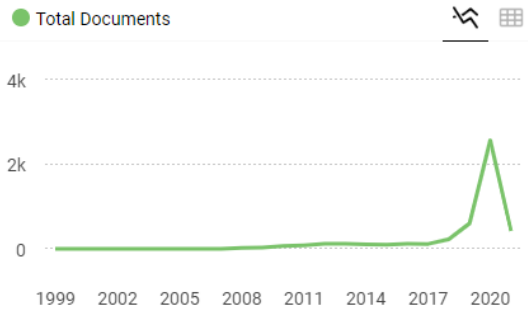
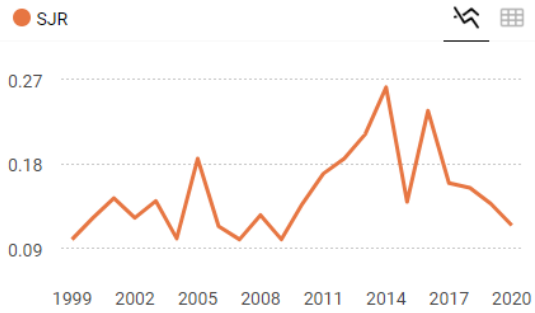
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"POTENSI HIDROGEL CARBOXYMETHYL CHITOSAN DENGAN EKSTRAK ETANOL KUNYIT (*Curcuma longa* L) SEBAGAI ALTERNATIF PERAWATAN LUKA INSISI PADA HEWAN COBA MENCIT (*Mus musculus*)"

*The Potency of Carboxymethyl Chitosan With Turmeric Extract (*Curcuma longa* L) as Wound Healing on Mice (*Mus Musculus*)*

PENELITI UTAMA :
David Setyo Budi

PEMBIMBING :
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2. Dr. Reny Tishom, M.Si

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DINYATAKAN LAIK ETIK.



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