

The Effect of Nanoliposome Turmeric Extract Gel (*Curcuma longa L.*) On TNF Alpha Level of Traumatic Ulcer Healing in The Labial Mucosa Wistar Rats (*Rattus Norvegicus*)

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Abstract. Background: Mucosal ulceration in the oral cavity is very common, especially ulcerations caused by trauma, which are commonly referred to as traumatic ulcers. Based on existing research, turmeric contains active substances in the form of curcumin and flavonoids to accelerate the wound healing process. The wound healing process is a very complex process consisting of 4 phases, namely hemostatic, inflammatory, proliferative, and remodeling phases. Macrophages will also release growth factor TNF alpha which triggers new tissue, after the inflammatory phase. Methods: This study was an experimental study using a randomized post-test only control group design with test animals of Ratus Novergicus. In this study, there were 9 groups, namely the control group, the 0.1% triamcinolone acetonide treatment group, and the turmeric extract nanoliposome gel group, each was given with 3 time series, namely the 3rd, 5th and 7th day. Then IHK preparations were made to see TNF alpha. Results: One way ANOVA test showed differences in TNF Alpha between the control group, the Triamcinolone acetonide, and nanoliposome gel group. TNF alpha shows a deflation amount from day 3, 5 and 7. Nanoliposome group treatment on day 7 had the lowest amount. It can be concluded that the nanoliposome gel of turmeric extract (*Curcuma longa L.*) has an effect on decreased TNF alpha levels. The decrease of tnf alpha numbers indicates that the inflammatory phase is over and continues to the proliferative and remodeling phase. Conclusion: Based on the results of this study, nanoliposome turmeric extract gel (*Curcuma longa L.*) has an effect decreased on TNF alpha levels.

1 Introduction

Traumatic ulcers are one of the most common diseases in the oral cavity caused by physical, thermal, chemical trauma and other triggers such as (bacteria, viruses, and fungi), immune system disorders, and deficiencies of certain food substances [1]. Traumatic ulcers can heal within a few days if they cause the cause, but if left untreated can lead to chronic ulcers [2].

The wound process, there is an increase in the level of tumor necrosis factor alpha (TNF alpha) which induces the release of intercellular adhesion molecule 1 (ICAM-1) which will increase the attachment of neutrophils to endothelial cells before entering the extravascular space or intracellular space. TNF alpha has several functions in

the inflammatory process, which can increase the pro-thrombotic role and stimulate adhesion molecules from leukocyte cells and induce endothelial cells, play a role in regulating macrophage activity and immune responses in tissues by stimulating growth factors and other cytokines, functioning as a regulator of hematopoietic as well as comitogens for T cells and B cells as well as the activity of neutrophil cells and macrophages [3].

Herbal plants have been widely used by the public as an alternative to treatment because these ingredients are easy to obtain, relatively inexpensive, and relatively low side effects [4]. Turmeric (*Curcuma longa L.*) is often used in the health world which functions as anti-inflammatory, antimutagenic, antioxidant, anticancer [5], antibacterial [6], and antiparasitic [7], can optimize and

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accelerate a healing. One of them is by using nanoparticles. Nanoliposomes have good biocompatibility and biodegradability so that they can improve bioactive performance by increasing bioavailability, in vivo and in vitro stability, and preventing unwanted interactions with other molecules [8].

Based on the above explanation, this study aimed to examine the effect of turmeric extract nanoliposome gel (*Curcuma longa L.*) on TNF alpha levels in the wound healing process in the form of traumatic ulcers on the labial mucosa of wistar rats.

2 Material and methods

This research has obtained worthy of UB's ethics with No. 069-KEP-UB-2020. This study used a *True Experimental method in vivo* using a *Randomized Posttest only Control Group Design*. Research conducted in the *Materia Medika Laboratory Batu, UB Biosciences, UMM Pharmacy, ITS Physics Department, Department of Chemistry, Faculty of Mathematics and Natural Sciences UGM*.

The study used 36 adult Wistar white rats as experimental animals. Furthermore, the experimental animals was divided into 9 groups, namely the control group on the 3rd day (K3), the control group on the 5th day (K5) and the control group on the 7th day (K7). For the triamcinolone acetonide treatment group, three time series (P3A, P5A, P7A) were also carried out and the treatment group with turmeric extract nanoliposome gel (P3B, P5B, P7B)

Group	Treatment
K3, K5 and K7	Control group
P3A, P5A and P7A	<i>Triamcinolone acetonide</i> group
P3B, P5B and P7B	Turmeric Extract <i>Nanoliposome</i> Gel group

2.1 Making Turmeric Extract (*Curcuma longa L.*)

Turmeric was extracted using maceration technique. Turmeric which has been weighed 1000 grams is then mashed using a blender and put into a measuring cup. The maceration process was repeated until the filtrate obtained was clear. Then evaporation of the solvent from the filtrate obtained using a rotary evaporator is carried out so that a more concentrated extract will be obtained.

The results of the turmeric extract were continued with the Liquid Chromatography and Mass Spectrometry (LC-MS) test which functions to identify the compounds present in the turmeric extract.

2.2 Nanoliposome Characterization Test of Turmeric Extract (*Curcuma longa L.*)

Nanoliposome extract then tested for characterization using TEM (Transmission Electron Microscopy) and PSA (Particle Size Analyzer) and Zeta Potential test. PSA

(*Particle Size Analyzer*) is used to measure the particle size distribution. Zeta potential test to determine the charge of nanoparticles using a zetasizer by diluting the sample with a dilution. The sample was put into a cuvette and analyzed at temperature.

2.3 Experimental Animal Treatment

Experimental animals were adapted for 7 days fed as much as 30 grams per day. After 7 days of adaptation, a traumatic ulcer was made on the labial mucosa of white rats with a cement stopper that had been heated until it was red hot to a depth . After 24 hours a traumatic ulcer will form, then Triamcinolone acetonide and gael nanoliposome turmeric extract (*Curcuma longa L.*) are administered. Then decapitation was carried out on the 3rd, 5th, and 7th day.

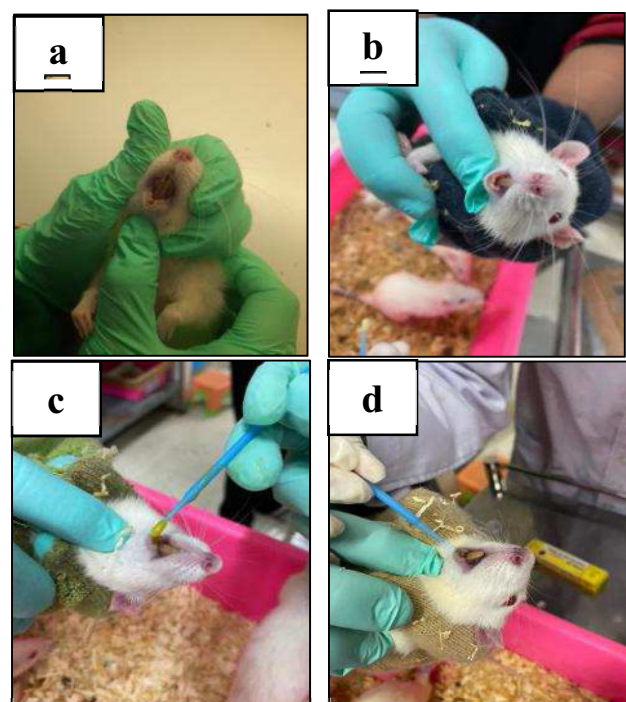


Figure 1. Making Traumatic Ulcer and Application Triamcinolone acetonide 0,1%, nanoliposome gel *Curcuma longa L.* (a) Experimental animals after making a traumatic ulcer with a cement stopper. (b) Experimental animals after 24 hours of ulceration. (c) Application of Triamcinolone acetonide. (d) Application of turmeric (*Curcuma longa L.*) extract nanoliposome gel.

2.4 Microscopic Observation

After that, preparations were made with HE (*hematoxylin-eosin*) staining and Immunohistochemistry. Furthermore, observations were made using an Olympus microscope. Epithelial thickness was measured using an ocular micrometer on the Image J software application with 400x magnification interpreted with five fields of view. TNF- α will appear with brownish spots on the cell nucleus of the preparations seen from 20 fields of view with 1000 times magnification.

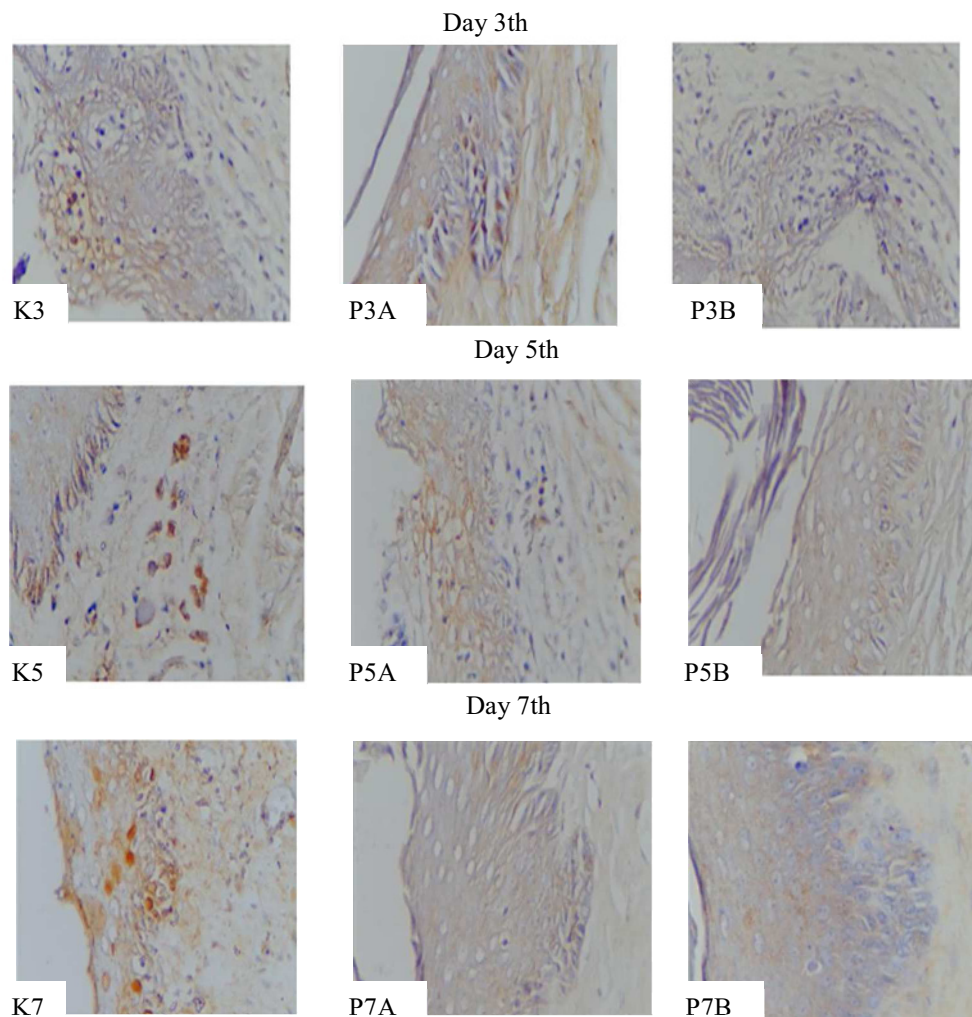


Figure 2. Result staining immunohistochemistry of TNF alpha. This observation uses a microscope with a magnification of 400x. K3: control group on the 3rd day; K5: control group on the 5th day; K7: control group on the 7th day; P3A: triamcinolone acetone treatment group on the 3rd day; P5A: triamcinolone acetone treatment group on the 5th day; P7A: triamcinolone acetone treatment group on the 7th day; P3B: turmeric extract nanoliposome gel on the 3rd day; P5B: turmeric extract nanoliposome gel on the 5th day; P7B: turmeric extract nanoliposome gel on the 7th day

3 Result and discussion

3.1 TNF Alpha Result

Based on the statistical test using the one way Anova test on day 3, there was a significant difference in the increase in the amount of TNF alpha with $P < 0.05$.

From the results of the post hoc TNF alpha test, there was a significant difference between the control group (K3) and the treatment group B (nanoliposome; P3B). But there was no significant difference between K3 and P3A, P3B and P3A.

The statistical results of the fifth day of research were also calculated using the same test, the one way Anova test. There were significant results in TNF alpha. The post hoc TNF alpha test showed that there were no significant differences between the P5A and P5B groups, while the comparison between the K5 and P5A groups and K5 and P5B groups had significant differences.

On the 7th day there was a significant difference on the ANOVA calculation and there was not significant difference on the Post Hoc Tukey calculation (cannot be

Table 1. TNF alpha level One Way Anova statistic in each group

Group		Total	Mean	Standard Deviation	P-value
Day 3rd	K3	41	14	2,517	0.036*
	P3A	35	12	2,000	
	P3B	27	9	2,646	
	Total	103	35	3,621	
Day 5th	K5	27	9	2,517	0.005*
	P5A	19	6	1,528	
	P5B	14	5	1,000	
	Total	60	20	3,742	
Day 7th	K7	21	7	2,000	0.024*
	P7A	12	4	2.517	
	P7B	8	2	1.528	
	Total	41	13	3,321	

understood), the administration of turmeric extract nanoliposome gel described the potential and effect on wound healing. Based on the one way ANOVA test on day 7, TNF alpha showed significant results. The control group (K7) and P7A, and the treatment group P7B and

P7A did not show a significant difference, while the control group K7 and P7B showed a significant difference. TNF alpha is also a major cytokine in the acute inflammatory response to Gram-negative bacteria and other microbes, but TNF alpha is not only a cytokine in the inflammatory response and also an indirect factor in increasing the process of angiogenesis with macrophages. When viewed from the results of the calculation of the presence of TNF alpha decreased. TNF alpha plays an important role in protecting wounds from infection, inducing fibroblast proliferation, keratinocytes and hair follicle regeneration [10].

Table 2. TNF alpha level Post Hoc statistic in each group

	Group	Mean Difference	Std. Error	P-Value
Day 3rd	K3 vs P3A	4.667	1.963	0.119
	K3 vs P3B	6.667	1.693	0.034*
	P3A vs P3B	2.000	1.963	0.593
Day 5th	K5 vs P5A	5.333	1.466	0.025*
	K5 vs P5B	7.667	1.466	0.005*
	P5A vs P5B	2.333	1.466	0.139
Day 7th	K7 vs P7A	4.333	1.678	0.092
	K7 vs P7B	6.333	1.678	0.002*
	P7A vs P7B	2.000	1.678	0.499

* Significant $p < 0.05$

TNF- α participates in vasodilatation and oedema formation, as well as leukocyte adhesion to the epithelium through expression of adhesion molecules. Increased levels of TNF- α are known to activate platelets, it regulates blood coagulation, contributes to oxidative stress at sites of inflammation [11]. That an increase in TNF levels will make the proliferation phase faster as well as wound healing. A decrease in the amount of TNF alpha indicates that the inflammatory phase is over and continues in the proliferative and remodeling phase.

4 Conclusion

Based on the results of this study, it can be concluded that the nanoliposome gel of turmeric extract (*Curcuma longa* L.) has an effect on decreased TNF alpha levels, and. The decrease of tnf alpha numbers indicates that the inflammatory phase is over and continues to the proliferative and remodeling phase

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