



Effectiveness of Turmeric Ethanol Extract (*Curcuma Longa*) in Accelerating Wound Healing After in Wistar Rats

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Abstract: RISKESDAS results in 2018 showed the DMFT (Decay, Missing and Filled Teeth Index of Indonesian people nationally 2.5% in 2013, rising to 57.6% in 2018. The main cells involved in the wound healing process are fibroblasts. Fibroblasts are stem cells that play a role in forming and putting fibers in the matrix, especially collagen fibers. Turmeric (*Curcuma longa*) has several pharmacological properties, including anti-inflammatory, anticancer, antioxidant, antiulcus, and antibacterial actions. The content of flavonoids in *Curcuma longa*, as an immunostimulating substance, then the production of growth hormones such as EGF, TGF α , PDGF, EGF, FGF, and TGF β will also increase, so that wound healing can be accelerated. This study was conducted to find out the effectiveness of turmeric ethanol extract (*Curcuma Longa*) in accelerating wound healing after tooth extraction. This study used 32 male Wistar rats divided into 2 groups, namely the treatment group given turmeric extract (*Curcuma Longa*) 45% and the treatment group given turmeric (*Curcuma Longa*) 90%. The wound healing process was assessed by observing fibroblast tissue on the 5th day after the extraction of Wistar mouse teeth. The results of the Chi-Square Test statistical analysis showed a significant difference in the number of fibroblasts in the wound healing process in Wistar rats after tooth extraction between the treatment group given turmeric extract (*Curcuma Longa*) 45% and the treatment group given Turmeric extract (*Curcuma Longa*) 90% after the 5th day with p-value ($0.032 < 0.05$). Turmeric extract (*Curcuma longa*) is 45% and 90% effective in speeding up the healing time of post-extraction wounds of Wistar mouse teeth. Turmeric extract (*Curcuma longa*) is 90% more effective than turmeric extract (*Curcuma longa*) 45% in speeding up wound healing time after extraction of rat teeth Wistar because of the flavonoid content in turmeric extract (*Curcuma longa*) 90% which helps accelerate wound healing higher than turmeric extract (*Curcuma longa*) 45%.

Keywords: tooth extraction; turmeric (*Curcuma Longa*); wound healing; fibroblasts

I. Introduction

Tooth extraction has a psychological impact on the patient, because the patient will lose his teeth (Siagian 2016). Riskesdas results in 2018 showed the DMFT (Decay, Missing and Filled Teeth) index of Indonesian people nationally 2.5% in 2013, rising to 57.6% in 2018 (Kementerian Kesehatan Republik Indonesia 2018). Tooth extraction will cause a wound in the form of alveolar bone that opens in the oral cavity. Physiologically, the body can repair damage to skin tissue (wounds) itself known as wound healing (Orongan and Siagian 2015). In a 2013 study conducted at FKG UNHAS Dental Hospital showed data on the prevalence of complications due to tooth extraction by 16.8% crown fractures, 13.6% root fractures, 4% dry sockets, 1.6% bleeding and pain by 1.6%. Prevalence of tooth extraction complications is bleeding by 4.54% and swelling by 2.27% (Lande, Kepel, and Siagian 2015). The wound healing process is divided into three main phases, namely, the inflammatory phase, the proliferation phase, and the remodeling phase (Budiman and Derrick 2013). The main cells involved in the wound healing process are fibroblasts. Fibroblasts are stem cells that play a role in forming and putting fibers in the

matrix, especially collagen fibers (Junquiera 2007). Herbal medicine products have been widely used since long ago until now in the medical world. Herbal remedies have been shown to be effective for health and do not cause too many side effects such as chemical drugs (Lone et al. 2018); (Amanah and Sadono 2017).

Turmeric (*Curcuma longa* Linn) has several pharmacological properties, including anti-inflammatory action (Manarin et al. 2019); (Setiadi, Khumaida, and Wahyuning Ardie 2017); (Kocaadam and Şanlier 2017), anticancer (Roihatul Mutiah 2015), antioxidant (Roihatul Mutiah 2015); (Abdul-Aziz 2011), and antibacterial. The content of flavonoids in *Curcuma longa*, as an immunostimulant substance, then the production of growth hormones such as EGF, TGF α , PDGF, VEGF, FGF, β will also increase, so that wound healing can be accelerated (Ayati et al. 2019). The purpose of this study was to find out the effectiveness of turmeric extract (*Curcuma longa*) 45% with turmeric extract (*Curcuma longa*) 90% in speeding up the healing time of post-tooth extraction wounds in Wistar rats.

II. Review of Literatures

Tooth extraction is the act of removing or moving teeth from the alveolus. Tooth extraction is also surgical action involving hard tissue and soft tissue from the oral cavity (Bakar 2014). Complications that are often encountered in tooth extraction include bleeding, swelling, pain, dry socket, fracture, etc (Chandra HM 2014). Bleeding can also occur due to the use of a drill that hits the mandibular canal (Pedersen 2012). Wound healing consists of a series of sequential processes. Wound healing can be divided into three phases: the inflammatory, proliferation, and remodeling phases, which are tissue re-processes (Sjamsuhidajat, R & Jong 2010). Fibroblasts are one of the healing components of wounds in the form of cells that are widely distributed in connective tissue, producing collagen substances, elastic fibers, and reticular fibers (Masir et al. 2012).

III. Research Methods

This study is an experimental laboratory study that uses a randomized controlled design with post-test only control group design patterns. This research will be conducted at the Laboratory of Pharmacology & Laboratory of Traditional Medicine Faculty of Pharmacy, University of North Sumatra, and Anatomical Pathology Laboratory of the Faculty of Medicine, the University of North Sumatra, which was conducted from January-March 2021. The sample used Wistar mice with Federer's formula so that the number of samples as many as 16 were given the treatment of turmeric extract 45% and 16 rats were given 90% turmeric extract. Tools used animal cages try, diagnostic sets, nierbeken, tooth extraction pliers (needle holders), sputts, gloves, masks, petri dishes jaw preparations, histological preparation manufacturing tools, microscopes. Ingredients used 45% turmeric extract, turmeric extract 90%, ketamine, formalin 10%, histological preparation ingredients with hematoxylin-eosin (HE) staining, 70% alcohol as sterilization ingredients, cotton pellets.

Removal of mouse teeth will be done using modifications of the needle holder under the anesthetic effect of ketamine 1000 mg / 10 ml dose of 20 mg/kg bb intraperitoneal. Every day the extraction of incisive teeth is 1 in every 5 rats. After tooth extraction, re-observe the extraction scar and give a tampon (cotton pellet) to stop bleeding in the wound for 5 minutes. On the 5th day after the extraction of teeth, mice from each group were sacrificed by physical means by dislocation of the neck, then taken the jaws of mice.

The tissue is fixated with 10% formalin for 24 hours at room temperature, then the decalcification process is carried out using a solution of Ethylene Diamine Tetra Acetic Acid (EDTA 10%) at room temperature. Then the process of dehydrating the tissues using alcohol. The next step of the embedding process (inserting the network into paraffin) and labeled / code. After the embedding stage is complete, the tissue is sliced in series with a thickness of approximately 6 microns using microtoms. The process of evaluating fibroblast cell response uses hematoxylin eosin (HE) coloring. The next stage, inserted into the solution xylol and object glass closed with deck glass and made observations using a light microscope. 14. Fibroblast density is assessed by calculating the number of fibroblasts at 5 points of view.

Histopathological scoring parameters for knowing the distribution of fibroblast tissues are carried out based on field of view are (Rizka, Budipramana, and Fauziah 2013):

1. (-) = No fibroblast tissue was found.
2. (+) = Slight number of fibroblasts (less than 10% per field of view)
3. (++) = Moderate fibroblast tissue (10%-50% per field of view)
4. (+++) = Large number of fibroblast tissue (50%-100% per field of view)

The data collected in this study is primary ⁵ta obtained from the results of measurements (score) on the histological picture of the process of accelerating wound healing after tooth extraction with the administration of turmeric extract (Curcumin Longa) 45% and turmeric extract (Curcumin Longa) 90%, on ¹⁶e 5th day after treatment. Analysis with the nonparametric Chi-Square Test, with a large value ($p < 0.05$) means that there are significant differences between groups.

IV. Discussion

⁷ 4.1 Distribution and Frequency Data of the Number of Fibroblasts per Field of View Post Tooth Extraction

²¹ **Table 1.** Distribution and Frequency Data on the Number of Fibroblasts per Field of View Post-Tooth Extraction

NO	Number of Fibroblasts	Turmeric (Curcuma Longa)			
		Concentration 45%	Concentration 90 %	n	%
1	No fibroblast tissue was found.	0	0	0	0
2	Slight number of fibroblasts (less than 10% per field of view)	9	28.1	2	6.2
3	Moderate fibroblast tissue (10%-50% per field of view)	4	12.5	7	21.9
4	The amount of fibroblastic tissue is large (50%-100% per field of view).	3	9.4	7	21.9

From the Table. 1 can be seen all samples found fibroblast tissue on the administration of Turmeric extract (Curcuma Longa) 45% and 90% post extraction of Wistar Mouse teeth. The number of fibroblasts found in the category is slight (less than 10% per field of view) in the administration of Turmeric extract (Curcuma Longa) 45% after the extraction of Wistar rats teeth as much as 9 (28.1%) tails and in the administration of Turmeric extract (Curcuma Longa) 90% as much as 2 (6.2%) tails. The number of

fibroblasts found in the moderate category (10%-50% per field of view) in the administration of Turmeric extract (Curcuma Longa) 45% after the extraction of Wistar Rat teeth as much as 4 (12.5%) tails and in the administration of Turmeric extract (Curcuma Longa) 90% as much as 7 (21.9%) tails. The number of fibroblasts found in many categories (50%-100% per field of view) in the administration of Turmeric extract (Curcuma Longa) 45% after extraction of Wistar Rat teeth as much as 3 (9.4%) tails and in the administration of Turmeric extract (Curcuma Longa) 90% as much as 7 (21.9%) tails.

4.2 Association of Fibroblast Tissue per Field of View in Wistar Rats Post Tooth Extraction with Administration of Turmeric Extract (Curcuma Longa) Concentration 45% and 90%

From the Table. 2 It can be seen that there is a significant relationship between the amount of fibroblast tissue per field of view in Wistar Rats post tooth extraction with the administration of Turmeric Extract (Curcuma Longa) with a concentration of 45% and Turmeric Extract (Curcuma Longa) concentration of 90%, $p = 0.032$ ($p < 0.05$).

Table 2. Association of Fibroblast Tissue Per Field of View in Wistar Rats Post Tooth Extraction with Administration of Aloe Vera Extract Concentrations of 45% and 90%

Number of Fibroblasts	Kunyit (Curcuma Longa)		
	Concentration 45%	Concentration 90 %	p
1. No fibroblast tissue was found.	0	0	
2. Slight number of fibroblasts (less than 10% per field of view)	9	2	
3. Moderate fibroblast tissue (10%-50% per field of view)	4	7	0,032*
4. The amount of fibroblastic tissue is large (50%-100% per field of view).	3	7	

Extraction of mouse teeth will be carried out under the anaesthetic effect of ketamine 1000 mg / 10 ml dose of 20 mg / kg bb intraperitoneal. After extraction, re-observe the wound after extraction and give tampons (cotton pellets) to stop bleeding in the wound for 5 minutes. Turmeric extract (Curcuma Longa) 45% was given to the treatment group I and Turmeric extract (Curcuma Longa) 90% in the treatment group II shortly after tooth extraction as much as 0.05 ml daily by dripping. The 5th day of the mouse jaw retrieval was then fixed with 10% formalin for 24 hours at room temperature, then the decalcification process was carried out using a solution of Ethylene Diamine Tetra Acetic Acid (EDTA 10%) at room temperature. Then dehydrate the tissues into a solution of toluol alcohol (1:1), using pure toluol. The process of evaluating fibroblast cell response ⁷ is hematoxylin eosin (HE) coloring. The density of fibroblasts is assessed by calculating the number of fibroblasts at 3 field of view. The sample test was conducted on the fifth day because fibroblasts are known to begin to grow during the third day to the seventh day of the wound healing process so researchers took an average of days that are on the fifth day (Masir et al. 2012).

From the results of this study obtained that all samples found fibroblast tissue in the administration of turmeric extract (Curcuma Longa) 45% and 90% post-extraction of Wistar mouse teeth. The number of fibroblasts found in the category is small (less than

10% per field of view) in the administration of Turmeric extract (Curcuma Longa) 45% post-extraction of rat teeth Wistar as much as 9 (28.1%) tails and in the administration of Turmeric extract (Curcuma Longa) 90% as much as 2 (6.2%) tails. The number of fibroblasts found in the moderate category (10%-50% per field of view) in the administration of Turmeric extract (Curcuma Longa) 45% after extraction of rat teeth Wistar as much as 4 (12.5%) tails and in the administration of Turmeric extract (Curcuma Longa) 90% as much as 7 (21.9%) tails.

The number of fibroblasts found in many categories (50%-100% per field of view) in the administration of Turmeric extract (Curcuma Longa) 45% post-extraction of rat teeth Wistar as much as 3 (9.4%) tails and in the administration of Turmeric extract (Curcuma Longa) 90% as much as 7 (21.9%) tails. Based on the analysis of Chi-Square data, there was a significant relationship between the amount of fibroblast tissue per field of view in Wistar rats after tooth extraction with the administration of Turmeric Extract (Curcuma Longa) 45% and Turmeric Extract (Curcuma Longa) 90%, $p = 0.032$ ($p < 0.05$). This is seen in the distribution of data on the number of fibroblasts that are large (50%-100% per field of view) in Turmeric (Curcuma Longa) 90% as much as 7 samples and in Turmeric (Curcuma Longa) 45% only 3 samples.

A small number of fibroblasts (less than 10% per field of view) is also found more in Turmeric (Curcuma Longa) 45% which is as many as 9 samples while in Turmeric (Curcuma Longa) 90% is only found as many₈ as 2 samples. The results of this study are supported by muthia et al research, (2019)₂₃ the effect of Turmeric (Curcuma Longa) on the closing time of a sore on the mucosa of the oral cavity of Wistar rats. The results of this study showed turmeric (Curcuma Longa) influenced the closing time of the sores on the mucosa of the oral cavity of Wistar rats. Wounds in Wistar rats given Turmeric (Curcuma Longa) are more quickly covered than Wistar mice that are not given Turmeric (Curcuma Longa) (Muthia Milasari 2019).

The results of this study are₁₂ in accordance with the research conducted by Fitriani (2014); Yunesa (2019), the results showed a significant difference between the control group and the treatment group on₅ the seventh day. This significant difference was seen from the amount of collagen fibrin in the control group compared₁₉ to the treatment group given Turmeric (Curcuma Longa). Turmeric (Curcuma Longa) plays an important role in stimulating the healing process of wounds. Turmeric (Curcuma Longa) stimulates the formation of new fibroblast cells and accelerates wound healing due to the glucomannan content, a complex polysaccharide that can stimulate fibroblasts to proliferate rapidly in the luk area (Fitriani 2014); (Yunesa, Gigi, and Utara 2019). Active substances such as manosa, glucomannan, chrysanthemum acid, acemannan, flavonoids, saponins, tannins, vitamin A, vit₁₀in C, vitamin E and enzymes contained in Turmeric (Curcuma Longa) are very helpful in the wound healing process.

According₁₁ to the results of the study Winarsih, dkk (2012), turmeric extract gel can reduce inflammation in the healing process of hyperglycemic back wounds (Win₂₄ih, Wientarsih, and Sutardi 2012). The enzyme content in Curcuma longa helps remove dead cells on the surface of the epidermis of the skin damaged by wounds and amino acids can help regenerate cells very quickly. The content of vitamin A in turmeric can stimulate collagen formation so that it triggers recapitalization. Vitamin A and Vitamin E can also speed up the recapitalization process by increasing blood flow to damaged₁₁s so that the process of restoring damaged epithelial cells is faster (Fitriani 2014). Vitamin C plays a role in cell differentiation, collagen synthesis, and increased proliferation of fibroblasts. In addition, vitamin C can also boost the immune system. This good immune state can improve the function of the immune₈ system, so it can increase proliferation. Saponins are steroids or triterpenoid glycosides that play an important role in

³ human and animal health. Saponins can trigger the vascular endothelial growth factor (VEGF) and increase the number of macrophages migrating to the wound area thereby increasing the production of cytokines that will activate fibroblasts in the wound tissue. Curcumin modulating the expression of TGF- β increases the formation of collagen, fibronectin, and proteoglycans and stimulates the proliferation of fibroblasts (Akbik et al. 2014); (Budiman et al. 2015).

Tannins contain astringents to stop bleeding, speed up wound healing and reduce mucous membrane inflammation and regenerate new tissue. In addition, tannin content has antibacterial abilities. Tannin content accelerates wound healing with several cellular mechanisms, namely cleaning free radicals and reactive oxygen, increasing wound closure, and increasing the formation of capillary blood vessels and fibroblasts. Flavonoids in turmeric (*Curcuma longa*) serve as antioxidants, antimicrobials, and also anti-inflammatory wounds. Flavonoids can help wound healing by increasing collagen formation, lowering tissue edema, and increasing the number of fibroblasts (Budiman et al. 2015).

The results showed the total level of flavonoids in Turmeric extract (*Curcuma Longa*) 90% is 2.39% and the total flavonoid content in Turmeric extract (*Curcuma Longa*) 45% is 1.19%, so turmeric extract (*Curcuma Longa*) is 90% more effective in speeding up wound healing. From the results of this study it can be seen that turmeric extract (*Curcuma Longa*) is 90% more effective in the wound healing process than Turmeric extract (*Curcuma Longa*) 45% because the higher the concentration of extracts, the higher the content in Turmeric extract (*Curcuma Longa*) is also higher, so the wound healing process is faster. Some of the difficulties in the study were Wistar mouse teeth that were easily fractured when extracted. This is due to the anatomy of the long Wistar mouse teeth in the socket and bent, so at the time of fracture, the researchers had to remove the rest of the teeth by slightly tearing soft tissue from the socket. ²² other difficulty that occurred during the study was to look for comparison substances to check vitamin C levels, so researchers did not check vitamin C levels and researchers only checked the total levels of flavonoids present in turmeric extract 45% by 90%.

V. Conclusion

Turmeric extract (*Curcuma longa*) is 45% and 90% effective in speeding up the healing time of post-extraction wounds of Wistar mouse teeth. Turmeric extract (*Curcuma longa*) is 90% more effective than turmeric extract (*Curcuma longa*) 45% in speeding up wound healing time after extraction of rat teeth wistar because of the flavonoid content in turmeric extract (*Curcuma longa*) 90% which helps accelerate wound healing higher than turmeric extract (*Curcuma longa*) 45%. Advice, more research is needed on the effectiveness of turmeric extract (*Curcuma longa*) 45% with 90% in speeding up the healing time of post-extraction wounds of Wistar rat teeth at different extract concentrations, different dosage forms, and with a larger number of research samples.

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