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Effectiveness Test of Turmeric Ethanol Extract (Curcuma Longa) in Accelerating Wound Healing after Tooth Extraction in Wistar Rats

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Abstract: **Turmeric (Curcuma longa Linn or Curcuma domestica Val) belongs to the Zingiberaceae family. People have long known it as a plant with many benefits, such as anti-inflammatory, anticancer, antioxidant, antiulcer, and antibacterial. This study aims to analyze the effectiveness of turmeric (Curcuma longa) extract 50% with 90% in accelerating wound healing time after tooth extraction in Wistar rats. This experimental laboratory study used a complete randomized design with a post-test-only control group design pattern. The experimental animals used in this study were 32 male Wistar rats, physically healthy, 2-3 months old, with body weight between 200-250 grams. The sample size was determined by the Federer formula, namely: (t - 1) (r - 1) ≥ 15, and the minimum sample size for each treatment was 16 rats. The results showed a significant relationship between the number of fibroblast tissue per visual field in Wistar rats after tooth extraction with the administration of Turmeric Extract (Curcuma Longa) 50% concentration and Turmeric Extract (Curcuma Longa) 100% concentration, p=0.008 (p<0.05). In conclusion, turmeric extract (Curcuma longa) 50% and 100% are effective in accelerating wound healing time after tooth extraction of Wistar rats. Turmeric extract (Curcuma longa) 100% is more effective than turmeric extract (Curcuma longa) 50% in accelerating wound healing time after tooth extraction of Wistar rats because the flavonoid content in turmeric extract (Curcuma longa) 100% which helps accelerate wound healing is higher than turmeric extract (Curcuma longa) 50%.**

Keywords: **Curcuma longa Linn, Wound healing, Tooth Extraction.**

I. INTRODUCTION

Tooth extraction will cause a wound in the form of exposed alveolar bone in the oral cavity. An injury is an anatomical damage or partial tissue destruction due to trauma. The severity of the wound depends on the amount of trauma received by the tissue. Physiologically, the body can repair damage to the skin tissue (injury), known as wound healing (1). Routine wound healing is a complex and dynamic process. The wound-healing process can be divided into three phases: inflammation, proliferation, and remodeling. These phases continue from the time of wounding until wound closure. The inflammatory phase is the body's reaction to the wound that starts after a few minutes and lasts about three days after the injury. The proliferation phase is characterized by the appearance of new blood vessels resulting from reconstruction and occurs within 3-24 days. Finally, the maturation phase is the final stage of the wound-healing process. This process takes more than one year, depending on the depth and extent of the wound (2).

The primary cells involved in the wound-healing process are fibroblasts. Fibroblasts are stem cells that form and lay down fibers in the matrix, especially collagen fibers. They secrete small tropocollagen molecules that combine with the primary

substance to form collagen fibers. Collagen will provide strength and integrity to any well-healed wound. In addition, fibroblasts more actively synthesize matrix components in response to the damage by proliferating and increasing fibrinogenesis. Therefore, fibroblasts are the leading agents in the wound-healing process (3). Herbal products have been used for a long time in the medical world. Nowadays, herbs are starting to be widely used for various treatments. Modern research results also show that herbal medicines are proven effective for health and do not cause side effects like chemical drugs (4).

Turmeric (Curcuma longa Linn or Curcuma domestica Val) belongs to the Zingiberaceae family. The public has long known it as a plant with many benefits, such as anti-inflammatory, anticancer, antioxidant, antiulcer, and antibacterial. In addition, the flavonoid content as an immunostimulant substance, the production of growth hormones such as EGF, TGFα, PDGF, VEGF, FGF, and TGFβ will also increase so that wound healing can be accelerated (5); (6); (7). Because of the above, the authors are interested in examining the effectiveness of turmeric extract (Curcuma longa) 50% with 90% in accelerating wound healing time after tooth extraction in Wistar rats.

II. RESEARCH METHODS

This experimental laboratory study uses a randomized controlled design with a post test only control group design pattern. This research will be conducted at the Pharmacology Laboratory & Traditional Medicine Laboratory, Faculty of Pharmacy, University of North Sumatra, and the Anatomical Pathology Laboratory, Faculty of Medicine, the University of North Sumatra, from September to December 2023. The experimental animals used in this study are Wistar rats, 32 males, physically healthy, 2-3 months old, with a body weight between 200-250 grams. The rats will be divided into two groups, namely, 16 treated with 50% turmeric extract (Curcumin Longa) and 16 treated with 100% turmeric extract (Curcumin Longa) to see the comparison of accelerated wound healing after tooth extraction. The sample size was determined by the Federer formula, namely: (t - 1) (r - 1) ≥ 15. Where t = several treatments; (2 treatments) r = several replications. Thus, the minimum sample size for each treatment was 16 rats.

= (t-1) (r-1) ≥15

= (2-1) (r-1) ≥15

= (r-1) ≥15

= (r-1) ≥15

= r ≥15 + 1

= r ≥16

Tools

Tools used in research :

1. Number-coded experimental animal cages.
2. Diagnostic set (mouth glass, sonde, tweezers).
3. Nierbeken.
4. Dental extraction forceps (in this case a needle holder is used) under sterile conditions.
5. Syringe.
6. Gloves.
7. Mask.
8. Petri dish of jaw preparation.
9. A set of tools for making histology preparations.
10. Microscope.

Material

Materials used in the study:

1. Turmeric (Curcumin Longa) Extract 50%
2. Turmeric Extract (Curcumin Longa) 100%
3. Ketamine.
4. Formalin 10%.
5. Histology preparation material with Hematoxylin Eosin (HE) staining.
6. 70% alcohol as sterilization material.
7. Cotton pellet.

Data Type

The type of data collected in this study is primary data obtained from the results of measurements (scoring) on the histological picture of the process of accelerating wound healing after tooth extraction by administering turmeric extract (Curcumin Longa) 50% and turmeric extract (Curcumin Longa) 100%.

Extraction on Turmeric (Curcumin Longa)

Collecting 3 kg of turmeric, the turmeric was washed and divided into two parts to take the inner flesh to obtain the gel. After washing, the turmeric flesh was dried in an incubator at 500 for 72 hours. The dried turmeric meat was then pulverized using a blender to form a powder. The turmeric meat that has become powder is then extracted by maceration with stirring. The extraction process uses water solvent. The powder is put into a maceration vessel or impermeable lid container and then filtered using filter paper; the pulp is macerated up to 2 times. The obtained maceration results were collected and evaporated using a rotary vacuum evaporator at 50oC until there was no more solvent condensation on the condenser. After the solvent is evaporated using a rotary vacuum evaporator, evaporation is continued using a 70-degree temperature water bath to obtain a pure extract. The turmeric extract was then diluted with water to get 50% and 100% extract concentrations.

Treatment of Wistar Rats

1. Before treatment, 32 rats were divided into 50% turmeric extract and 100% turmeric extract. After that, all rats were adapted for one week. Then, animals were put into cages, with five rats in each cell in the same environmental conditions, given the same food, and monitored for health.

2. Rat tooth extraction will be performed using a modified needle holder under the anesthetic effect of ketamine 1000 mg/10 ml at a dose of 20 mg/kg bw intraperitoneally.

3. One incisor tooth will be extracted from every five rats daily.

4. After tooth extraction, observe the extraction wound and apply a tampon (cotton pellet) to stop bleeding in the wound for 5 minutes.

5. Dropped turmeric extract (Curcumin Longa) 50% in treatment group I and dropped turmeric extract (Curcumin Longa) 100% in treatment group II shortly after tooth extraction as much as 0.05 ml every day.

6. After extraction and treatment, the test animals (rats) were fed fine porridge with attention to the health of the test animals.

7. On the 5th day after tooth extraction, rats from each group were physically sacrificed by neck dislocation. The rat's tail was held and then placed on a surface it could reach. The rat will stretch its body; when the rat's body extends, a holder held by the left hand is placed on the nape of the neck. The right hand pulls the tail hard so the rat's neck will be dislocated. Then the jaw of the rat is taken out.

8. Then the tissue was fixed with 10% formalin for 24 hours at room temperature, then the decalcification process was carried out using Ethylene Diamine Tetra Acetic Acid (EDTA 10%) solution at room temperature.

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9. Tissue dehydration was then performed using alcohol. First, the specimen was put into toluol alcohol solution (1:1) using pure toluol, then into a paraffin-saturated toluol solution.

10. The following process is infiltration in the oven by inserting the specimen into liquid paraffin.

11. The embedding process is carried out (inserting the tissue into paraffin) and then labeled/coded. After the embedding stage, the tissue is sliced in series with a thickness of approximately 6 microns using a microtome.

12. Evaluating fibroblast cell response using Hematoxylin Eosin (HE) staining. The procedure that must be done is deparaffinization using xylol and alcohol solution, then continued with the rehydration process with alcohol. After that, it is washed with running water, rinsed with distilled water, and then wiped. The glass slide was then placed in Meyer's hematoxylin solution, washed with running water, and then rinsed with distilled water, after which the staining was assessed under a light microscope. If the staining has been considered good, proceed to the next step, namely the dehydration process with alcohol in stages, and then wipe.

13. The next step, put it into xylol solution, and the object glass was covered with deck glass and observed using a light microscope.

14. Fibroblast density was assessed by counting the fibroblasts in 5 fields of view.

Histopathology Scoring Parameters for Fibroblast Counts

Histopathology scoring parameters to determine the distribution of fibroblast tissue is done based on the field of view is:

1. (-) = no fibroblast tissue found
2. (+) = small number of fibroblasts (less than 10% per field of view)
3. (++) = moderate amount of fibroblast tissue (10%-50% per field of view)
4. (+++) = large amount of fibroblast tissue (50%-100% per field of view) 4.

Data Analysis Method

Data analysis using the SPSS 16 program. Research using a pure experiment with a nonparametric Chi-Square Test, after testing, showed that ($p < 0.05$) means there is a significant difference between groups.

III. RESULTS AND DISCUSSION

Table 1. Distribution and Frequency Data of Fibroblast Tissue Counts Per Field of View After Tooth Extraction

NO	Number of Fibroblasts	Turmeric (Curcuma Longa)			
		Concentration 50%		Concentration 100%	
		n	%	n	%
1	No fibroblast tissue found	0	0	0	0
2	A small number of fibroblasts (less than 10% per field of view)	9	28	2	6
3	Moderate amount of fibroblast tissue (10%-50% per field of view)	4	13	7	22
4	A large amount of fibroblast tissue (50%-100% per field of view).	3	9	7	22

From Table 1. it can be seen that all samples found fibroblast tissue in the administration of turmeric extract (Curcuma Longa) 50% and 100% after tooth extraction of Wistar rats. The number of fibroblasts found in the small category (less than 10% per field of view) in the administration of turmeric extract (Curcuma Longa) 50% after tooth extraction of Wistar rats was 9 (27.4%) and in the administration of turmeric extract (Curcuma Longa) 100% was 2 (6.2%). The number of fibroblasts found in the moderate category (10%-50% per field of view) in the administration of turmeric extract (Curcuma Longa) 50% after tooth extraction of Wistar rats as many as 4 (12.5%) heads and in the administration of turmeric extract (Curcuma Longa) 100% as many as 7 (21.9%) heads. The number of fibroblasts found in the large category (50% - 100% per field of view) in the administration of turmeric extract (Curcuma Longa) 50% after tooth extraction of Wistar rats as many as 3 (9.4%) heads and in the administration of turmeric extract (Curcuma Longa) 100% as many as 7 (21.9%) heads.

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Table 2. Relationship between the number of tissue fibroblasts per field of view in Wistar rats after tooth extraction with turmeric extract concentrations of 50% and 100%

Number of Fibroblasts	Turmeric (Curcuma Longa)		p
	Concentration 50%	Concentration 100%	
1. No fibroblast tissue was found	0	0	0,008*
2. A small number of fibroblasts (less than 10% per field of view)	8	3	
3. Moderate amount of fibroblast tissue (10%-50% per field of view)	6	8	
4. A Large fibroblast tissue (50%-100% per field of view).	2	6	

Significant p<0.05. Chi Square Test

From Table 2. it can be seen that there is a significant relationship between the number of fibroblast tissue per field of view in Wistar rats after tooth extraction by administering Turmeric Extract (Curcuma Longa) with a concentration of 50% and Turmeric Extract (Curcuma Longa) with a concentration of 100%, p = 0.008 (p <0.05).

IV. DISCUSSION

Tooth extraction is the process of removing both whole teeth and remaining roots from the alveolar because it cannot be treated anymore. Tooth extraction will cause injury by exposing the alveolar bone in the oral cavity. Wounds are anatomical or partial tissue damage due to trauma (1). The body will repair tissue damage (harm), known as the wound healing process, and begins from injury until wound closure. The primary cells involved in the wound-healing process are fibroblasts. The proliferation of fibroblasts determines the outcome of wound healing. This is because fibroblasts will produce collagen that will link the wound and affect the revitalization process that will close the wound.

This study aims to compare the effectiveness of 50% Turmeric (Curcuma Longa) extract and 100% Turmeric (Curcuma Longa) extract in accelerating wound healing time after tooth extraction of Wistar rats. The samples used in this study were Wistar rats. Wistar rats are known to have a physiological body similar to human physiology and have a short average age of 1-2 years, so it is appropriate to be used as an experimental object. The number of research samples taken was 32 Wistar rats that were physically healthy and 2-3 months old with body weight between 200-250 grams. The samples were divided into two groups, namely 16 (50%) for the group treated with 50% Turmeric (Curcuma Longa) extract and 16 (50%) for the group treated with 100% Turmeric (Curcuma Longa) extract. R rat teeth will be extracted under the anesthetic effect of ketamine 1000 mg/10 ml dose of 20 mg/kg bw intraperitoneally. After extraction, the post-extraction wound will be observed, and a tampon (cotton pellet) will be applied to stop bleeding in the damage for 5 minutes. 50% Turmeric (Curcuma Longa) extract was given to treatment group I and 100% Turmeric (Curcuma Longa) extract to treatment group II shortly after tooth extraction as much as 0.05 ml daily by drip. On the 5th day, the rat jaw was taken and fixed with 10% formalin for 24 hours at room temperature. The decalcification process used Ethylene Diamine Tetra Acetic Acid (EDTA 10%) solution at room temperature. The tissue was then dehydrated in a toluol alcohol solution (1:1) using the pure tool (8).

The fibroblast cell response evaluation process used Hematoxylin Eosin (HE) staining. Fibroblast density was assessed by counting the number of fibroblasts in 3 fields of view. The sample test was conducted on the fifth day because fibroblasts are known to start growing during the third to the seventh day of the wound healing process, so researchers took the average day, namely on the fifth day (Stojanovic et al., 2011). From the results of this study, it was found that all samples found fibroblast tissue in the administration of 50% and 100% Turmeric (Curcuma Longa) extract after tooth extraction of Wistar rats. The number of fibroblasts located in the small category (less than 10% per field of view) in the administration of turmeric extract (Curcuma Longa) was 50% after tooth extraction of Wistar rats was 9 (28.1%). In the administration of turmeric extract (Curcuma Longa), 100% was 2 (6.2%). The number of fibroblasts found in the moderate category (10%-50% per field of view) in the administration of turmeric extract (Curcuma Longa) 50% after tooth extraction of Wistar rats as many as 4 (12.5%) and in the administration of turmeric extract (Curcuma Longa) 100% as many as 7 (21.9%) (9).

The number of fibroblasts found in the large category (50%-100% per field of view) in the administration of turmeric extract (Curcuma Longa) was 50% after tooth extraction of Wistar rats as many as 3 (9.4%) and in the administration of turmeric extract (Curcuma Longa) 100% as many as 7 (21.9%). Based on Chi-Square data analysis, there is a significant relationship

between the number of fibroblast tissue per field of view in Wistar rats after tooth extraction with the administration of 50% Turmeric Extract (Curcuma Longa) and 100% Turmeric Extract (Curcuma Longa), p=0.032 (p<0.05). This is seen in the distribution of data on the number of fibroblasts that are many (50%-100% per field of view) in Turmeric (Curcuma Longa), 100% as many as seven samples, and in Turmeric (Curcuma Longa), 50% only three pieces.

The number of fibroblasts that were small (less than 10% per field of view) was also found to be more in Turmeric (Curcuma Longa) 50%, namely nine samples, while in Turmeric (Curcuma Longa) 100% only found as many as two pieces. The results of this study are supported by research by Ruauw et al. in 2016 on the effect of Turmeric (Curcuma Longa) on the closure time of incision wounds on the oral mucosa of Wistar rats. This study showed that Turmeric (Curcuma Longa) influences the closure time of incision wounds on the oral mucosa of Wistar rats. Wounds in Wistar rats given Turmeric (Curcuma Longa) closed faster than in Wistar rats that were not given Turmeric (Curcuma Longa) (Ruauw et al., 2016). The results of this study are also by research conducted by Arijani E and Khoswanto C in 2008 on using 100% Turmeric (Curcuma Longa) as a modulator of collagen density in wounds after guinea pig incisor tooth extraction (Cavia cobaya).

The results showed a significant difference between the control and treatment groups on the seventh day. This significant difference was seen in the amount of collagen fibrin in the control group compared to the treatment group given Turmeric (Curcuma Longa). The content of Turmeric (Curcuma Longa) is essential in stimulating the wound healing process. Turmeric (Curcuma Longa) promotes the formation of new fibroblast cells and accelerates wound healing due to glucomannan content. This complex polysaccharide can stimulate fibroblasts to proliferate rapidly in the wound area. In addition, active substances such as mannose, glucomannan, chrysophane acid, acemannan, flavonoids, saponins, tannins, vitamin A, vitamin C, vitamin E, and enzymes contained in Turmeric (Curcuma Longa) are beneficial in the wound healing process (10).

Saponins are steroids or triterpenoid glycosides that are essential to human and animal health. Saponins can trigger 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 wound tissue. Saponins will increase the action of TGF-β on fibroblast receptors, and TGF-β will stimulate fibroblast migration and proliferation. Tannin contains astringents to stop bleeding, accelerate wound healing, reduce mucous membrane inflammation, and regenerate new tissue. In addition, tannin has an antibacterial ability. Tannin content accelerates wound healing with several cellular mechanisms, namely cleaning free radicals and reactive oxygen, increasing wound closure, and forming capillary blood vessels and fibroblasts (Kusumawardhani et al., 2015). Flavonoids in Turmeric (Curcuma Longa) function as antioxidants, antimicrobials, and anti-inflammatories in wounds. Flavonoids can help wound healing by increasing collagen formation, reducing tissue edema, and increasing the number of fibroblasts (Mawarti and Ghofar, 2014). The results showed the total flavonoid content in 100% Turmeric (Curcuma Longa) extract was 2.39%, and the entire flavonoid content in 50% Turmeric (Curcuma Longa) extract was 1.19%, so it was found that 100% Turmeric (Curcuma Longa) extract was more effective in accelerating wound healing (11).

From the results of this study, it can be seen that 100% Turmeric (Curcuma Longa) extract is more effective in the wound healing process than 50% Turmeric (Curcuma Longa) extract because the higher the concentration of the section, the higher the content in the Turmeric (Curcuma Longa) extract so that the wound healing process is faster. However, some difficulties in this study are the teeth of Wistar rats that easily fracture when pulled. This is because the anatomy of the Wistar rat teeth is long in the socket and crooked, so when the fracture, the researcher must remove the remaining teeth by slightly tearing the soft tissue from the socket. Another difficulty during the study was finding a comparator substance to check vitamin C levels, so the researcher did not check vitamin C levels and only checked the total flavonoid levels in the turmeric extract 50% with 100%.

V. CONCLUSION

Based on the results and discussions that have been carried out in this study, it can be concluded:

1. Turmeric extract (Curcuma longa) 50% and 100% are effective in accelerating wound healing time after tooth extraction of Wistar rats.

2. Turmeric extract (Curcuma longa) 100% is more effective than turmeric extract (Curcuma longa) 50% in accelerating wound healing time after tooth extraction of Wistar rats because the flavonoid content in turmeric extract (Curcuma longa) 100%, which helps accelerate wound healing is higher than turmeric extract (Curcuma longa) 50%.

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