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ANALYSIS OF THE EFFECTIVENESS OF ARECA NUT EXTRACT IN WOUND HEALING AFTER TOOTH EXTRACTION IN WISTAR RATS

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ABSTRACT

One of the plants that is often used by the community for wound healing is areca nut (Areca catechu L) which is spread throughout Indonesia. Areca nut (Areca catechu L) seeds contain phytochemical compounds that are beneficial for wound healing such as antioxidants, anti-inflammatory, and antibacterial compound. The purpose of this study was to analyse the effectiveness of Areca nut (Areca catechu L.) seed extract in accelerating wound healing time after tooth extraction. This experimental laboratory study uses a randomized controlled design with a post-test only control group design pattern. 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 60% Areca nut (Areca catechu L) and 16 treated with 120% Areca nut (Areca catechu L) to see the comparison of accelerated wound healing after tooth extraction. Data analysis using the SPSS 16 program, research using a pure experiment with a non-parametric Chi-Square Test, after testing, showed that (p <0.05) means there is a significant difference between groups. Results there is a significant relationship between the number of fibroblast tissue per field of view in Wistar rats after tooth extraction by giving Areca nut extract (Areca catechu L.) with a concentration of 60% and Areca nut extract (Areca catechu L.) concentration of 120%%, p = 0.001 (p <0.05). concluded that areca nut extract (Areca catechu L.) 60% and 120% are effective in accelerating wound healing time after tooth extraction of Wistar rats.

KEYWORDS: ***Areca nut, tooth extraction, wound healing.***

I. INTRODUCTION

The process of tooth extraction will cause injury to the area of the extracted tooth (1); (2). There are two techniques of tooth extraction, namely, simple technique and surgical technique (3). The simple technique is more common than the surgical technique, while the surgical technique is only performed if the simple technique cannot be performed. Treatment used as wound healing or infected with bacteria often uses antibiotics because it can kill microbes quickly (4); (5). Continuous use of antibiotics can cause resistance in bacteria. Natural ingredients, especially medicinal plants, can be an alternative to healing wounds or infections (6). The wound healing process after tooth extraction consists of 5 overlapping stages, consisting of blood clot formation, granulation tissue, preosseous tissue, bone trabeculae and epithelialisation. In the early stages of the healing process, a blood clot is formed that fills the empty socket, the blood clot is formed from blood cells and fibrin tissue (1). One of the plants that is often used by the community for wound healing is areca nut (Areca catechu L) which is spread throughout Indonesia (7). Areca nut (Areca catechu L) seeds contain phytochemical compounds that are beneficial for wound healing such as antioxidants, anti-inflammatory, and antibacterial compounds (8); (9). These compounds include polyphenol (20%), fat (15%,) fibre (20%), and alkaloids (10); (11). The purpose of this study was to analyse the effectiveness of Areca nut (Areca catechu L.) seed extract 60% with 120% in accelerating wound healing time after tooth extraction.

II. RESEARCH METHODS

This experimental laboratory study uses a randomized controlled design with a post-test only control group design pattern. 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 60% Areca nut (Areca catechu L) and 16 treated with 120% Areca nut (Areca catechu L) 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.

$= (t-1)\ (r-1) \geq 15$
$= (2-1)\ (r-1) \geq 15$
$= (r-1) \geq 15$
$= (r-1) \geq 15$
$= r \geq 15 + 1$
$= r \geq 16$

Materials used in the study, Areca nut (Areca catechu L) Extract 60%, Areca nut (Areca catechu L) Extract 120%, Ketamine, Formalin 10%, histology preparation material with Hematoxylin Eosin (HE) staining, 70% alcohol as sterilization material, cotton pellet. 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

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administering Areca nut (Areca catechu L) 60% and Areca nut (Areca catechu L) 120%.

Collecting 3 kg of Areca nut (Areca catechu L), the Areca nut (Areca catechu L) was washed and divided into two parts to take the inner meat to obtain the gel. After washing, the flesh of the Areca nut (Areca catechu L) was dried in an incubator at 500 ℃ for 72 hours. The dried flesh of the Areca nut (Areca catechu L) was then pulverized using a blender until it became powder. Areca nut (Areca catechu L) meat that had become powder was then extracted by maceration while stirring. The extraction process uses a water solvent. The powder was put into a maceration vessel or container with a watertight lid and then filtered using filter paper; the pulp was macerated up to 2 times. The obtained maceration results were collected and evaporated using a rotary vacuum evaporator at a temperature of 500C until there was no more solvent condensation on the condenser. After the solvent was evaporated using a rotary vacuum evaporator, the evaporation was continued using a 70°C water bath to obtain a pure extract. The Areca nut (Areca catechu L) extract was then diluted with water to get 50% and 100% extract concentrations. Before treatment, 32 rats were divided into 50% Areca nut extract and 100% Areca nut 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.

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. One incisor tooth will be extracted from every five rats daily. After tooth extraction, observe the extraction wound and apply a tampon (cotton pellet) to stop bleeding in the wound for 5 minutes. Dropped Areca nut (Areca catechu L) 50% in treatment group I and dropped Areca nut (Areca catechu L) 100% in treatment group II shortly after tooth extraction as much as 0.05 ml every day. After extraction and treatment, the test animals (rats) were fed fine porridge with attention to the health of the test animals. 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. 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.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. The following process is infiltration in the oven by inserting the specimen into liquid paraffin. 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. 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. The next step, put it into xylol solution, and the object glass was covered with deck glass and observed using a light microscope. Fibroblast density was assessed by counting the fibroblasts in 5 fields of view. Histopathology scoring parameters to determine the distribution of fibroblast tissue is done based on the field of view is:

(-) = no fibroblast tissue found

(+) = small number of fibroblasts (less than 10% per field of view)

(++) = moderate amount of fibroblast tissue (10%-50% per field of view)

(+++) = large amount of fibroblast tissue (50%-100% per field of view) 4.

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

III. RESULT AND DISCUSSION

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

Number of Fibroblasts	Extract Areca nut *(Areca catechu L.)*			
	Concentration 60%		Concentration 120%	
	n	%	n	%
No fibroblast tissue found	0	0	0	0
Small number of fibroblasts (less than 10% per field of view)	9	28	3	6
Moderate amount of fibroblast tissue (10%-50% per field of view)	5	13	5	19
Large amount of fibroblast tissue (50%-100% per field of view).	2	9	8	25

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Table 1 It can be seen that all samples found fibroblast tissue in the administration of Areca nut extract (Areca catechu L.) 60% and 120%% 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 Areca nut extract (Areca catechu L.) 60% after tooth extraction of Wistar rats was 9 (27.4%) and in the administration of Areca nut extract (Areca catechu L.) 120%% as many as 2 (6.2%). The number of fibroblasts found in the moderate category (10%-60% per field of view) on the administration of Areca nut extract (Areca catechu L.) 60% after tooth extraction of Wistar rats was 5 (12.5%) and on the administration of Areca nut extract (Areca catechu L.) 120%% as many as 5 (19%). The number of fibroblasts found in the large category (60%-120% per field of view) in the administration of Areca nut extract (Areca catechu L.) 60% after tooth extraction of Wistar rats as many as 3 (9.4%) and in the administration of Areca nut extract (Areca catechu L.) 120%%% as many as 8 (25.2%).

Table 2.Relationship between the number of tissue fibroblasts per field of view in Wistar rats after tooth extraction with 50% and 100% concentration of Areca nut extract (Areca catechu L.)

Number of Fibroblasts	Extrac Areca nut *(Areca catechu L.)*		p
	Concentration 60%	Concentration 120%	
No fibroblast tissue found	0	0	**0,001***
Small number of fibroblasts (less than 10% per field of view)	9	3	
Moderate amount of fibroblast tissue (10%-50% per field of view)	5	5	
Large amount of fibroblast tissue (50%-100% per field of view).	2	8	

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 giving Areca nut extract (Areca catechu L.) with a concentration of 60% and Areca nut extract (Areca catechu L.) concentration of 120%%, p = 0.001 (p <0.05).

This study aims to determine the comparison of the effectiveness of 60% Areca nut extract and 120% Areca nut 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 use as an experimental object (Lailani et al., 2013). The number of research samples taken was 32 wistar rats that were physically healthy and 2-3 months old with body weight between 200-260 grams. The samples were divided into two groups, 16 (60%) for the group treated with 60% areca nut extract and 16 (60%) for the group treated with 120% areca nut extract.

Extraction of rat teeth will be carried out under the anaesthetic effect of ketamine 1200 mg/10 ml dose of 20 mg/kg bw intraperitonially. After extraction, the post-extraction wound will be observed and a tampon (cotton pellet) will be applied to stop bleeding in the wound for 5 minutes. 60% areca nut extract was given to treatment group I and 120% areca nut extract to treatment group II shortly after tooth extraction as much as 0.05 ml daily by dropping. On the 5th day, rat jaws were taken and fixed with 10% formalin for 24 hours at room temperature, followed by decalcification using Ethylene Diamine Tetra Acetic Acid (EDTA 10%) solution at room temperature. The tissue was then dehydrated in toluol alcohol solution (1:1), using pure toluol.

The fibroblast cell response evaluation process used Hematoxylin Eosin (HE) staining. Fibroblast density was assessed by counting the number of fibroblasts in 3 field of view. The sample test was carried out on the fifth day because fibroblasts are known to start growing during the third to the seventh day of the wound healing process, so the researchers took the average day, namely on the fifth day fibroblast tissue was found in the administration of areca nut (Areca catechu L.) extract 60% and 120%% 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 Areca nut extract (Areca catechu L.) 60% after tooth extraction of Wistar rats was 9 (27.4%) and in the administration of Areca nut extract (Areca catechu L.) 120%% as many as 2 (6.2%). The number of fibroblasts found in the moderate category (10%-60% per field of view) on the administration of Areca nut extract (Areca catechu L.) 60% after tooth extraction of Wistar rats was 5 (12.5%) and on the administration of Areca nut extract (Areca catechu L.) 120%% as many as 5 (19%). The number of fibroblasts found in the large category (60%-120% per field of view) in the administration of Areca nut extract (Areca catechu L.) 60% after tooth extraction of Wistar rats as many as 3 (9.4%) and in the administration of Areca nut extract (Areca catechu L.) 120%%% as many as 8 (25.2%).

The results of this study found that there was a significant relationship between the number of fibroblast tissue per field of view in Wistar rats after tooth extraction by giving areca nut extract (Areca catechu L.) with a concentration of 60% and areca nut extract (Areca catechu L.) concentration of 120%%, p = 0.001 (p <0.05). The effect of areca nut seeds on the closure time of incision wounds on the oral mucosa of wistar rats. The results of this study showed that areca nut seeds have an influence on the closure time of incision wounds on the oral mucosa of Wistar rats. Wounds in wistar rats that were given areca nut seeds closed faster than wistar rats that were not given areca

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nut seeds. The results of this study are also in accordance with research conducted by Arijani E and Khoswanto C in 2008 on the use of areca nut seeds as a modulator of collagen density in post-extraction wounds of guinea pig incisor teeth (Cavia cobaya) (1); (12). The results showed a significant difference between the control group and the treatment group on the seventh day. This significant difference was seen from the amount of collagen fibrin in the control group compared to the treatment group given areca nut seeds. The content of areca nut plays an important role in stimulating the wound healing process. Areca nut serves to stimulate the formation of new fibroblast cells and accelerate wound healing due to the content of glucomannan, a complex polysaccharide that can stimulate fibroblasts to proliferate rapidly in the wound area. Also supported by Handayani (2017), states that ethanol extract of areca nut at concentrations of 20%, 40% and 60% has an effect as a burn medicine. 20% ethanol extract with a wound healing percentage of (84.33%), 40% concentration (87.67%), and 60% concentration (89.67%). The concentration of areca nut ethanol extract group that has optimum activity on burn wound healing is 60% concentration (8).

IV. CONCLUSION

Based on the results and discussions that have been carried out in this study, it can be concluded that areca nut extract (Areca catechu L.) 60% and 120% are effective in accelerating wound healing time after tooth extraction of Wistar rats.

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