*International Journal of Research in Engineering and Science (IJRES)*
*ISSN (Online): 2320-9364, ISSN (Print): 2320-9356*
*www.ijres.org Volume 11 Issue 4 ǁ April 2023 ǁ PP. 176-181*

# Effect of Giving Extract of Areca Seeds in Accelerating Wound Healing After Tooth Extraction in Wistar Rats

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***Abstract***
*Areca nut (Areca catechu L) seeds contain phytochemical compounds beneficial for wound healing, such as antioxidants and anti-inflammatory and antibacterial compounds. These compounds include polyphenols (20%), fat (15%), fiber (20%), and alkaloids. (4); (5); (6). Based on the above, the author is interested in examining the effectiveness of areca nut (Areca catechu L.) extract 50% with 100% 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 rats will be divided into two groups, namely, 16 rats treated with 50% areca nut (Areca catechu L) and 16 rats treated with 100% areca nut (Areca catechu L) to see the comparison of accelerated wound healing after tooth extraction. The study used a pure experiment with a non-parametric Chi-Square Test after the test showed that ($p<0.05$) means there is a significant difference between groups. The results of a significant relationship between the number of fibroblast tissue per visual field in Wistar rats after tooth extraction by giving areca nut extract (Areca catechu L.) concentration of 50% and areca nut extract (Areca catechu L.) concentration of 100%, $p=0.002$ ($p<0.05$). The conclusion is that areca nut extract (Areca catechu L.) 50% and 100% effectively accelerates wound healing time after tooth extraction in Wistar rats. Areca nut extract (Areca catechu L.) 100% is more effective than areca nut extract (Areca catechu L.) 50% in accelerating wound healing time after tooth extraction in Wistar rats because the flavonoid content in areca nut extract (Areca catechu L.) 100% which helps accelerate wound healing is higher than areca nut extract (Areca catechu L.) 50%.*

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

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Date of Submission: 01-04-2023 Date of acceptance: 12-04-2023

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## I. INTRODUCTION

Tooth extraction is the procedure of removing a tooth and its root from the alveolar bone socket using forceps, elevators or a transalveolar approach (surgery) (1). There are two techniques for tooth extraction, namely, the simple technique and the surgical technique. The simple technique is performed more often than the surgical technique, while the surgical technique is only performed if the simple technique cannot be performed (2). The tooth extraction process will cause a wound in the area of the extracted tooth. An injury is a condition of tissue loss or disconnection. When a blood vessel is severed, the damaged platelets will release several components such as histamine, bradykinin, serotonin, and prostaglandins, and the body will react to the wound, which is a complex and complex dynamic wound-healing process.

The treatment used for wound healing or infection with bacteria often uses antibiotics because they can kill microbes quickly. However, continuous use of antibiotics can cause resistance in bacteria. Natural ingredients, especially medicinal plants, can be an alternative to cure wounds or infections (3). Indonesia is an area with a variety of biodiversity, including flora diversity. For example, Areca nut (Areca catechu L) seeds contain phytochemical compounds beneficial for wound healing, such as antioxidants and anti-inflammatory and antibacterial compounds. These compounds include polyphenols (20%), fat (15%,) fiber (20%), and alkaloids. (4); (5); (6). Because of the above, the authors are interested in examining the effectiveness of Areca nut (Areca catechu L.) extract 50% with 100% 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. 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% Areca nut (Areca catechu L) and 16 treated with 100% 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) ≥15
= (2-1) (r-1) ≥15
= (r-1) ≥15
= (r-1) ≥15
= r ≥15 + 1
= r ≥16

***Tools***

Tools used in research :
Number-coded experimental animal cages.
Diagnostic set (mouth glass, sonde, tweezers).
Nierbeken.
Dental extraction forceps (in this case a needle holder is used) under sterile conditions.
Syringe.
Gloves.
Mask.
Petri dish of jaw preparation.
A set of tools for making histology preparations.
Microscope.

***Material***

Materials used in the study:
Areca nut (Areca catechu L) Extract 50%
Areca nut (Areca catechu L) Extract 100%
Ketamine.
Formalin 10%.
Histology preparation material with Hematoxylin Eosin (HE) staining.
70% alcohol as sterilization material.
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 Areca nut (Areca catechu L) 50% and Areca nut (Areca catechu L) 100%.

***Extraction on Areca nut (Areca catechu L)***

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.

***Treatment of Wistar Rats***

1. 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.
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 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.
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.
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:

(-) = 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 Method***

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 50% | | Concentration 100% | |
| | n | % | n | % |
| No fibroblast tissue found | 0 | 0 | 0 | 0 |
| Small number of fibroblasts (less than 10% per field of view) | 8 | 25 | 2 | 6 |
| Moderate amount of fibroblast tissue (10%-50% per field of view) | 5 | 16 | 5 | 16 |
| Large amount of fibroblast tissue (50%-100% per field of view). | 3 | 9 | 9 | 28 |

Table 1 shows that all samples found fibroblast tissue in the administration of 50% and 100% Areca nut (Areca catechu L.) extracts 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 50% Areca nut (Areca catechu L.) extract after tooth extraction of Wistar rats was 8 (25%). In administering 100% Areca nut (Areca catechu L.), the ethe quote was 2 (6%). The number of fibroblasts found in the moderate category (10%-50% per field of view) in the

administration of 50% Areca nut (Areca catechu L.) extract after tooth extraction of Wistar rats was 5 (16%). In the administration of 100% Areca nut (Areca catechu L.), the esectionwas 5 (16%). The numbness of fibroblasts found in the large category (50% - 100% per field of view) in the administration of Areca nut extract (Areca catechu L.) 50% after tooth extraction for Wistar rats was 3 (9%). In administering Areca nut extract (Areca catechu L.), 100% was 9 (28%).

**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.)* | | |
|---|---|---|---|
| | Concentration 50% | Concentration 100% | p |
| No fibroblast tissue found | 0 | 0 | **0,002*** |
| Small number of fibroblasts (less than 10% per field of view) | 8 | 2 | |
| Moderate amount of fibroblast tissue (10%-50% per field of view) | 5 | 5 | |
| Large amount of fibroblast tissue (50%-100% per field of view). | 3 | 9 | |

*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 Areca nut extract (Areca catechu L.) with a concentration of 50% and Areca nut extract (Areca catechu L.) with a concentration of 100%, p = 0.002 (p <0.05).

Tooth extraction is a procedure to remove the tooth and its root from the alveolar bone socket using forceps, elevators, or a trans alveolar approach (surgery). Before tooth removal, a local anesthetic consisting of lidocaine mixed with epinephrine can be given to where the action will be performed. There are two tooth extraction techniques: simple design and surgical technique. The simple design is performed more often than the surgical technique, while the surgical procedure is only performed if the simple process cannot be performed.

The wound healing process after tooth extraction consists of 5 overlapping stages: blood clot formation, granulation tissue, preosseous tissue, bone trabeculae, and epithelialization. In the early stages of the healing process, a blood clot fills the empty socket; the blood clot is formed from blood cells and fibrin tissue. The blood clot then matures, creating granulation tissue rich in new blood vessels and inflammatory cells such as neutrophils, macrophages, lymphocytes, and fibroblast infiltrates. Granulation tissue forms on the second and third day after tooth extraction and completely replaces the blood clot on the seventh day. After that, granulation tissue is gradually replaced with temporary connective tissue or preosseous tissue.

The body will repair damaged tissue (wounds), known as the wound healing process, and begins from the time of the injury until wound closure (Novyana and Susianti, 2016). 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 (Masir et al., 2012).

This study aims to compare the effectiveness of 50% Areca nut extract and 100% 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 a short average age of 1-2 years, so it is appropriate to use them as experimental objects. 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 16 (50%) for the group treated with 50% Areca nut extract and 16 (50%) for the group treated with 100% Areca nut extract.

Rat tooth extraction will be performed under the anesthetic effect of ketamine 1000 mg/10 ml at a 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. Areca nut extracts 50% was given to treatment group I and 100% to treatment group II shortly after tooth extraction as much as 0.05 ml daily by dropping. On the 5th day, the rats' jaws were 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 toluol alcohol solution (1:1) using a pure tool.

The fibroblast cell response evaluation process used Hematoxylin Eosin (HE) staining. Fibroblast density was assessed by counting the number of fibroblasts in 3fieldsd of view. The sample test was carried out on the fifth day because fibroblasts are known to start growing on the third day to the seventh day of the wound healing process, so researchers took the average day, namely on the fifth day. From the results of this study, it was obtained that all samples found fibroblast tissue in the administration of 50% and 100% Areca nut 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 50% Areca nut extract after tooth extraction of Wistar rats was 9 (28.1%,) and in the administration of 100% Areca nut extract was 2 (6.2%). The number of fibroblasts found in the moderate category

(10%-50% per field of view) in the administration of 50% Areca nut extract after tooth extraction of Wistar rats was 4 (12.5%,) and in the administration of 100% Areca nut extract was 7 (21.9%).

The number of fibroblasts found in the large category (50%-100% per field of view) in the administration of 50% Areca nut extract after tooth extraction of Wistar rats was 3 (9.4%,) and in the administration of 100% Areca nut extract was 7 (21.9%). Based on Chi-Square data analysis, there was a significant relationship between the number of fibroblast tissue per field of view in Wistar rats after tooth extraction with the administration of 50% Areca nut extract and 100% Areca nut extract, p=0.032 (p<0.05). This was seen in the data distribution of the fibroblasts (50%-100% per field of view) in Areca nut 100% as many as seven samples and in Areca nut 50% only three pieces.

Jumlah fibroblas yang sedikit (kurang dari 10% per lapangan pandang) juga ditemukan lebih banyak pada Areca nut 50% yaitu sebanyak 9 sampel sedangkan pada Areca nut 100% hanya ditemukan sebanyak 2 sampel. Hasil penelitian ini didukung oleh penelitian Ruauw dkk. tahun 2016 tentang pengaruh Areca nut terhadap waktu penutupan luka sayat pada mukosa rongga mulut tikus wistar. Hasil peneltian ini menunjukkan Areca nut memiliki pengaruh terhadap waktu penutupan luka sayat pada mukosa rongga mulut tikus wistar. Luka pada tikus wistar yang diberi Areca nut lebih cepat tertutup dibandingkan tikus wistar yang tidak diberikan Areca nut (Ruauw dkk., 2016). Hasil penelitian ini juga sesuai dengan peneltian yang dilakukan oleh Arijani E dan Khoswanto C tahun 2008 tentang penggunaan Areca nut 100% sebagai modulator dari densitas kolagen pada luka paska ekstraksi gigi insisivus marmut (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 that was given Areca nut. The Areca nut's content is essential in stimulating the wound healing process. Areca nut promotes the formation of new fibroblast cells and accelerates wound healing due to glucomannan content. In addition, this complex polysaccharide can stimulate fibroblasts to increase quickly in the wound area.

This research is supported by Jane (2015), with the research title The Effect of Giving Areca nut Extract (Areca Catechu L.) on Bleeding Time After Tooth Extraction in Wistar Male Rats (Rattus Norvegicus L.). The results showed that the average bleeding time of the treatment group was 0.6125 (minutes. seconds). The mean bleeding time of the treatment group was not much different from the mean bleeding time of the positive control group given transmit, which was 0.3475 (min. sec) and shorter than the negative control group of 96% ethanol with a mean bleeding time of 1.79 (min. sec). The research that has been done concludes that there is an effect of giving Areca nut extract (Areca catechu L.) at a dose of 13.834 mg / 200 g BW to the bleeding time after tooth extraction in male Wistar rats. In addition to accelerating bleeding time, Areca nut extract can accelerate wound healing. This is due to the compounds in Areca nut that have astringent, chronic anti-inflammatory, antimicrobial, and antioxidative activities (7).

Handayani's research (2017) stated that Areca nut ethanol extract at concentrations of 20%, 40%, and 60% has an effect as a burning medicine. 20% ethanol extract with a percentage of wound healing amounted to (84.33%), 40% concentration (87.67%), and 60% concentration (89.67%). The attention of the Areca nut ethanol extract group that has optimum activity against burn wound healing is 60% (5). Asfi's research (2021) shows that ethanol extract from pine seeds can accelerate the healing of cuts in rabbits which is maximized with a concentration of 7%. Furthermore, the results of the ANOVA statistical test on rabbit cuts using Areca nut extract obtained a significance value of P> 0.05 (at the α level of 0.05, namely 0.000 < 0.005). This means that each treatment provides a meaningful difference (significant); for this, it is necessary to continue testing the average difference of each treatment with the LSD Post Hoc tests (4).

From the results of this study, it can be seen that 100% Areca nut extract is more effective in the wound healing process than 50% Areca nut extract because the higher the concentration of the section, the higher the content in the Areca nut extract so that the wound healing process is faster. However, some difficulties in this study were that the teeth of Wistar rats were easily fractured when extracted. This is due to the anatomy of the Wistar rat teeth, which are long in the socket and crooked, so when the fracture occurs, 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%.

## IV. CONCLUSION

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

1. Areca nut (Areca catechu L.) extracts of 50% and 100% effectively accelerate wound healing time after tooth extraction in Wistar rats.
2. Areca nut extract (Areca catechu L.) 100% is more effective than areca nut extract (Areca catechu L.) 50% in accelerating wound healing time after tooth extraction of Wistar rats because the flavonoids content in areca nut extract (Areca catechu L.) 100% which helps accelerate wound healing is higher than areca nut extract (Areca catechu L.) 50%.

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