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Evaluation of the effect of areca nut seed extract in facilitating wound healing after tooth extraction in wistar rats

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Abstract
One of the plants commonly used by the community for wound healing is the betel nut (Areca catechu L.), distributed widely throughout Indonesia. Betel nut (Areca catechu L.) contains phytochemical compounds beneficial for wound healing, such as antioxidants, anti-inflammatory, and antibacterial agents. This study aims to evaluate the effectiveness of betel nut (Areca catechu L.) seed extract in accelerating the healing process after tooth extraction. This laboratory experimental research used a completely random design with a post-test-only control group design. Thirty-two healthy male Wistar rats, aged 2-3 months and weighing 200-250 grams, were used as research subjects. The rats were divided into two groups: 16 rats received treatment with 60% betel nut (Areca catechu L.) fruit extract, and 16 rats received treatment with 120% betel nut (Areca catechu L.) fruit extract to compare the acceleration of wound healing after tooth extraction. Data analysis was performed using SPSS 21, and the study adopted a pure experimental method with a non-parametric Chi-Square Test. After the test, the results showed that ($p < 0.05$), indicating a significant difference between the groups. The study results demonstrated a significant relationship between the number of fibroblast tissues per field of view in Wistar rats after tooth extraction with the administration of betel nut (Areca catechu L.) fruit extract at concentrations of 60% and 120%, with a p-value of 0.001 ($p < 0.05$).

Keywords: Areca nut, tooth extraction, wound healing

Introduction

Certainly, tooth extraction involves the physical removal of a tooth or its remaining roots from the alveolar socket, which results in injury to the surrounding area. The extraction creates an open wound in the oral cavity, where the tooth was located, leading to anatomical damage or destruction of the tissue due to the trauma caused by the extraction process. The body initiates a wound-healing process to repair the damaged tissue and restore the affected area after tooth extraction (Putri, 2020) [9]; (Enur, 2021) [3]. Tooth extraction procedures encompass two primary techniques: simple extraction and surgical extraction. The simple extraction technique is typically employed for visible and easily accessible teeth. Dentists use specialized forceps to grasp and remove the tooth, and the procedure generally involves minimal trauma to the surrounding tissues.

On the other hand, the surgical extraction technique is utilized in more complex cases, such as impacted or partially erupted teeth. This method may require an incision in the gum tissue or removing bone around the tooth to facilitate extraction. Surgical extraction is commonly employed when dealing with fractured teeth or those that haven't fully erupted. The choice between these techniques depends on factors such as the tooth's position, condition, and the overall complexity of the extraction process, with both methods aiming to ensure the safe and effective removal of the tooth while minimizing trauma to surrounding tissues (Fitriani, 2014) [4].

The simple extraction technique is more commonly employed than the surgical technique in routine dental procedures. Typically, dentists opt for the simple approach when the tooth is visible and accessible and can be safely removed using forceps. The surgical technique, on the other hand, is reserved for more complex cases where the tooth is impacted, fractured, or not fully erupted. It comes into play when the straightforward approach of the simple technique is not feasible (Sorongan & Siagian, 2015) [11]; (HM, 2014). The prolonged and excessive use of antibiotics poses the risk of bacteria developing resistance, a significant concern in healthcare. To address this issue, natural ingredients, particularly medicinal plants, offer an alternative approach to healing wounds or managing infections. Medicinal plants often contain bioactive compounds with antimicrobial properties, providing a natural source for combating bacterial infections. These compounds may include essential oils, flavonoids, and other phytochemicals known for their therapeutic effects.

Embracing natural alternatives aligns with the growing interest in herbal and plant-based remedies, emphasizing their potential benefits in wound healing and infection management. Utilizing medicinal plants can contribute to diversifying treatment options, reducing reliance on antibiotics, and potentially mitigating the development of antibiotic resistance. However, it's crucial to note that the effectiveness of natural remedies may vary, and consultation with healthcare professionals is advisable for comprehensive and personalized treatment plans (Puspita Dewi *et al*., 2013) [8]. 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 (Putri, 2020) [9]. One of the plants often used by the community for wound healing is areca nut (Areca catechu L), which is spread throughout Indonesia (Jane *et al*., 2015) [7]. Areca nut (Areca catechu L) seeds contain phytochemical compounds that are beneficial for wound

healing, such as antioxidants, anti-inflammatory, and antibacterial compounds (Handayani *et al*., 2017) [5]; (Asfi & Yulianti, 2021) [2]. These compounds include polyphenol (20%), fat (15%,) fiber (20%), and alkaloids (Afni *et al*., 2015) [1]; (Rairisti, 2014) [10]. This study aimed to analyze the effectiveness of Areca nut (Areca catechu L.) seed extract 50% with 100% in accelerating wound healing time after tooth extraction.

Research Methods

This experimental laboratory study adopts a randomized controlled design with a post-test-only control group design pattern. The research involves using Wistar rats, precisely 32 physically healthy males aged 2-3 months, weighing 200-250 grams. The rats will be systematically divided into two groups, with 16 individuals in each group. One group will receive treatment with a 50% Areca nut (Areca catechu L), while the other group will be treated with a 100% Areca nut (Areca catechu L). The primary objective is to compare the effects of these different concentrations on wound healing acceleration after tooth extraction.

The determination of the sample size adheres to the Federer formula, which states that (t - 1) (r - 1) should be greater than or equal to 15, where 't' represents the number of treatments (2 treatments in this case), and 'r' signifies the number of replications. Consequently, each treatment group's calculated minimum sample size is established at 16 rats. This rigorous methodology ensures statistical validity and reliability in assessing the impact of 50% and 100% Areca nut concentrations on the wound healing process in Wistar rats after tooth extraction.

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

The materials utilized in this study include Areca nut (Areca catechu L) Extract at concentrations of 50% and 100%, ketamine for anesthesia, Formalin 10% for fixation, histology preparation materials featuring Hematoxylin Eosin (HE) staining, 70% alcohol for sterilization, and cotton pellets. The primary data for this research is gathered through measurements (scoring) based on histological images, explicitly focusing on the accelerated wound healing process following tooth extraction under the administration of Areca nut (Areca catechu L) at concentrations of 50% and 100%.

Obtaining Areca nut (Areca catechu L) extract involved collecting 3 kg of Areca nuts, washing them, and separating the inner meat for gel extraction. After washing, the meat was dried in an incubator at 50℃ for 72 hours and then pulverized into powder using a blender. The powdered Areca nut (Areca catechu L) flesh underwent extraction by maceration with a water solvent. The resulting maceration was filtered, and the pulp underwent this process twice. The collected maceration results were evaporated using a rotary vacuum evaporator at 50℃ until no solvent condensation occurred on the condenser. Further evaporation took place using a 70°C water bath to obtain a pure extract. This extract was then diluted with water to achieve 50% and 100% concentrations.

Before treatment, 32 rats were divided into groups for 50% and 100% Areca nut extract administration. Following this, all rats underwent a one-week adaptation period. Subsequently, the animals were placed in cages, with five rats in each cell, exposed to the same environmental conditions, provided identical food, and monitored for their health throughout the study. This detailed methodology ensures the standardized preparation and administration of Areca nut extracts in a controlled experimental setting.

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. I dropped Areca nut (Areca catechu L) 50% in treatment group I. I dropped Areca nut (Areca catechu L) 100% in treatment group II as much as 0.05 ml every day shortly after tooth extraction. 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 carried 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, and the decalcification process was carried out using an ethylene diamine tetraacetic acid (EDTA 10%) solution at room temperature. Tissue dehydration was then performed using alcohol.

First, the specimen was put into a toluol alcohol solution (1:1) using pure toluol and 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. It evaluated 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, flushed 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 was to 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:

(-) = 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.

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 was found	0	0	0	0
A small number of fibroblasts (less than 10% per field of view)	8	50%	2	13%
Moderate amount of fibroblast tissue (10%-50% per field of view)	4	25%	5	31%
A large amount of fibroblast tissue (50%-100% per field of view).	4	25%	9	56%

Table 1 shows that all samples found fibroblast tissue in the administration of areca nut extract (Areca catechu L.) 50% and 100% after extraction of Wistar rat teeth. At initial observation, no fibroblast tissue was detected in any group receiving areca nut extract at 50% or 100% concentrations. In the second group, fibroblasts slightly increased (less than 10% per field of view). A total of 8 samples (50%) at a concentration of 50% and two models (13%) at a concentration of 100% showed the presence of fibroblast tissue. Furthermore, in the third group, the number of fibroblasts was seen moderately (10%-50% per field of view). Four samples (25%) at 50% concentration and five (31%) at 100% concentration indicated moderate fibroblast tissue. In the last group, the fourth group, there appeared to be many fibroblasts (50%-100% per field of view). Four samples (25%) at a concentration of 50% and nine samples (56%) at a concentration of 100% showed an increase in fibroblast tissue. Thus, the results of this study indicated that giving areca nut seed extract at a concentration of 100% tended to have a more positive influence on increasing the number of fibroblasts when compared to a concentration of 50%.

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 50% and Areca nut extract (Areca catechu L.) concentration of 100%%, p = 0.004 (p <0.05).

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

The extraction of rat teeth will be performed under the anesthetic effect of ketamine at a dose of 20 mg/kg body weight, administered intraperitoneally with a concentration of 1200 mg/10 ml. Following the tooth extraction, the post-extraction wound will be carefully observed, and a tampon (cotton pellet) will be applied to the wound site to control bleeding. This tampon will be left in place for 5 minutes. The treatment procedure involves the administration of 50% Areca nut extract to treatment group I and 100% Areca nut extract to treatment group II immediately after tooth extraction. The administration will be carried out daily, with a dosage of 0.05 ml applied by dropping. On the 5th day post-extraction, rat jaws will be collected and fixed with a 10% formalin solution for 24 hours at room temperature. Subsequently, the decalcification process will be carried out using an ethylene diamine tetraacetic acid (EDTA 10%) solution at room temperature. The tissue will then undergo dehydration in a toluol alcohol solution (1:1), utilizing pure toluol. This comprehensive protocol outlines the steps involved in the tooth extraction procedure, the application of Areca nut extract treatments, and the subsequent collection and preparation of rat jaws for further analysis.

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 50%	Concentration 100%	
No fibroblast tissue was found	0	0	0,004*
A small number of fibroblasts (less than 10% per field of view)	8	2	
Moderate amount of fibroblast tissue (10%-50% per field of view)	4	5	
A large amount of fibroblast tissue (50%-100% per field of view).	4	9	

Significant p<0.05. Chi-Square Test

The evaluation of fibroblast cell response in this study utilized Hematoxylin Eosin (HE) staining. Fibroblast density was determined by counting the number of fibroblasts in three fields of view. The sample test was conducted on the fifth day since fibroblasts are known to initiate growth between the third and seventh days of the wound-healing process. The researchers chose the fifth day as the average day for assessing fibroblast tissue in Wistar rats after tooth extraction and administering 50% and 100% concentration of areca nut extract (Areca catechu L.). The study results revealed a significant relationship between the number of fibroblast tissues per field of view in Wistar rats after tooth extraction with the administration of areca nut extract (Areca catechu L.) at concentrations of 50% and 100%, with a p-value of 0.001 (p < 0.05). The research further explored the impact of areca nut seeds on the closure time of incision wounds in the oral mucosa of Wistar rats. It was observed that wounds in Wistar rats treated with areca nut seeds closed faster than those in rats without areca nut seed treatment.

These findings align with a study by Arijani E and Khoswanto C 2008, which investigated the use of areca nut seeds as a modulator of collagen density in post-extraction wounds of guinea pig incisor teeth (Cavia cobaya). The collective results support the notion that areca nut extract, particularly at different concentrations, influences the fibroblast response and accelerates wound closure in the oral mucosa of Wistar rats after tooth extraction (Putri, 2020) [9]; (Sugiaman, 2011) [12]. The results showed a significant difference between the control and treatment groups on the seventh day. The observed significant difference in wound healing between the control group and the treatment group receiving areca nut seeds is evident from the increased amount of collagen fibrin in the treatment group. The content of areca nut plays a crucial role in stimulating the wound healing process by promoting the formation of new fibroblast cells and accelerating wound closure. This stimulatory effect is attributed to glucomannan, a complex polysaccharide found in areca nut, which can prompt the rapid proliferation of fibroblasts in the wound area.

These findings are consistent with the research by Handayani (2017) [5], which supports the wound-healing properties of the ethanol extract of areca nut. The study demonstrated that areca nut extract at concentrations of 20%, 40%, and 50% had a positive impact as a treatment for burns. Specifically, the 20% ethanol extract exhibited a wound healing percentage of 84.33%, the 40% concentration showed 87.67%, and the 50% concentration demonstrated the highest efficacy with a wound healing percentage of 89.67%. This suggests that the 50% concentration of areca nut ethanol extract is the most effective in promoting burn wound healing, providing additional evidence for the beneficial effects of areca nut in wound healing processes (Handayani *et al*., 2017) [5].

Conclusion

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

1. Betel nut seed extract (Areca catechu L.) is 50% and 100% effective in accelerating wound healing time after extraction of Wistar rat teeth.
2. Areca catechu L. Areca nut extract (Areca catechu L.) is 100% more effective than areca nut extract (Areca catechu L.) 50% in accelerating wound healing time after wistar rat tooth extraction due to the flavonoid content in areca nut seed extract (Areca catechu L.) 100% that helps accelerate wound healing is higher than betel nut extract (Areca catechu L.) 50%.

References

1. Afni N, Said N, Yuliet Y. Uji Aktivitas Antibakteri Pasta Gigi Ekstrak Biji Pinang (Areca Catechu L.) Terhadap Streptococcus Mutans Dan Staphylococcus Aureus. Jurnal Farmasi Galenika (Galenika Journal of Pharmacy) (E-Journal),2015:1(1):48–58. Https://Doi.Org/10.22487/J24428744.2015.V1.I1.7900
2. Asfi D, Yulianti S. Uji Aktivitas Ekstrak Etanol Biji Pinang (Areca Catechu L.) Terhadap Penyembuhan Luka Sayat Kelinci (Oryctolagus Cuniculus) Arief. Jurnal Kesehatan Yamasi Makasar,2021:5(2):121–127.
3. Enur. Gambaran pencabutan gigi tetap berdasarkan jenis kelamin di Puskesmas satuan pemukiman 4 Prafi Manokwari Papua Barat Enur[1], Jeana Lydia Maramis[2], Novaritha Koch[3],2021:4(1):25–29.
4. Fitriani D. Pengaruh Ekstrak Kunyit Terhadap Peningkatan Jumlah Makrofag Pada Soket Pasca Pencabutan Gigi Cavia Cobaya, 2014, 1–4.
5. Handayani F, Sundu R, Karapa HN. Uji Aktivitas Ekstrak Etanol Biji Pinang (Areca Catechu L.) Terhadap Penyembuhan Luka Bakar Pada Kulit Punggung Mencit Putih Jantan (Mus musculus). Jurnal Ilmiah Manuntung,2017:2(2):154. https://doi.org/10.51352/jim.v2i2.60
6. HM C. Buku Petunjuk Praktis Pencabutan Gigi (1st ed). Sagung Seto, 2014.
7. Jane W, Bernat H, Wellsy L. Pengaruh Pemberian Ekstrak Biji Pinang (Areca Catechu L.) Terhadap Waktu Perdarahan Pasca Ekstraksi Gigi Pada Tikus Jantan Wistar (Rattus Norvegicus L.). Journal Ilmiah Sains,2015:15(2):25–28.
8. Puspita Dewi I, Damriyasa I, Anom Dada I. Bioaktivitas Ekstrak Daun Tapak Dara (Catharanthus Roseus) Terhadap Periode Epitelisasi Dalam Proses Penyembuhan Luka Pada Tikus Wistar. Indonesia Medicus Veterinus,2013:2(1):58–75.
9. Putri GA. Pada Penyembuhan Luka Soket Pasca Pencabutan Gigi Tikus Putih Galur Wistar (Rattus Novergicus) Secara Hematoxilin Eosin (He) Universitas Sumatera Utara Medan, 2020.
10. Rairisti A. Uji Aktivitas Ekstrak Etanol Biji Pinang (Areca catechu L.). Naskah Publikasi,2014:1(1):1–24.
11. Sorongan RS, Siagian KV. Efektivitas Perasan Daun Pepaya Terhadap Aktivitas Fibroblas Pasca Pencabutan Gigi Pada Tikus Wistar Jantan. Pharmacon,2015:4(4):52–57. https://doi.org/10.35799/pha.4.2015.10192
12. Sugiaman VK. Peningkatan Penyembuhan Luka di Mukosa Oral Melalui Pemberian Aloe Vera (Linn.) Secara Topikal Topical. Maranatha Journal of Medicine and Health,2011:11(1):70–79.