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Review of the effectiveness of andaliman fruit extract (Zanthoxylum Piperitum) in healing wounds after tooth extraction in wistar rats

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Abstract
Andaliman contains phenols, monoterpenes, sesquiterpenes, nones, and essential oils belonging to the group of terpenoid compounds. Based on its chemical composition and physiological activity, the use of andaliman can be increased in various fields, including spices, preservatives, drugs and supplements, and vegetable pesticides. This study aimed to evaluate the effectiveness of andaliman fruit extract (Zanthoxylum piperitum) at concentrations of 50% and 100% in the wound healing process after tooth extraction in rats. Experimental research in this laboratory used a randomized design complete with a post-test-only control group design pattern. This study used 32 male white rats of the Wistar strain who were physically healthy, aged 2-3 months, and weighed between 200-250 grams. These rats were divided into two groups where 16 received treatment with 50% Andaliman fruit extract (Zanthoxylum Piperitum) and 16 others received treatment with 100% Andaliman fruit extract (Zanthoxylum Piperitum). This group division aims to compare the acceleration of wound healing after tooth extraction. The research method used was purely experimental, and statistical analysis was performed with a non-parametric Chi-Square Test after the test showed that ($p<0.05$), indicating a significant difference between groups. The results showed that there was a significant relationship between the number of fibroblast tissues per field of view in Wistar rats after tooth extraction with the administration of Andaliman Fruit Extract (Zanthoxylum piperitum) concentration of 50% and Andaliman Fruit Extract (Zanthoxylum piperitum) concentration of 100%, with a value of $p = 0.003$ ($p < 0.05$). Based on the results and discussion of the study, it can be concluded that Andaliman Fruit Extract (Zanthoxylum piperitum) at concentrations of 50% and 100% is effective in accelerating the healing time of wounds after tooth extraction in Wistar rats.

Keywords: Andaliman fruit, wound healing, depilation

Introduction

Tooth extraction leads to a wound characterized by an exposed alveolar bone within the oral cavity (Sorongan & Siagian, 2015) [10]. An injury, on the other hand, refers to anatomical damage or partial destruction of tissue caused by trauma, with the severity of the wound contingent upon the extent of trauma inflicted on the tissue. Physiologically, the body can mend damage to its skin tissue, referred to as wound healing (Putri, 2020) [5]. This therapeutic process encompasses three primary phases: the inflammatory phase, the proliferation phase, and the remodeling phase, persisting from the initiation of the wound until its closure.

The inflammatory phase, a response to the damage, commences shortly after injury and spans approximately three days (Afni *et al*., 2015) [1]. Subsequently, the proliferation phase ensues, marked by the emergence of new blood vessels through reconstruction, transpiring within 3-24 days. The maturation phase represents the concluding stage of the wound-healing process (Jane *et al*., 2015) [4]. Andaliman bears significant cultural relevance to the Batak tribe, particularly in North Sumatra, where it is frequently employed as a spice in traditional Batak cuisine. Beyond its culinary role, andaliman harbors terpenoid substances with antioxidant, antimicrobial, and immunostimulant effects. Silalahi (2021) [8] indicates that andaliman encompasses phenol compounds, monoterpenes, sesquiterpenes, nones, and essential oils, classifying them as terpenoid compounds. Leveraging its chemical composition and physiological attributes, andaliman can transcend its role as a mere seasoning, finding utility as a preservative, medicinal material, supplement, and vegetable pesticide (Silalahi & Lumbantobing, 2021) [8].

Numerous studies underscore the multifaceted potential of andaliman, portraying it as an antimicrobial, antioxidant, anti-inflammatory, xanthine oxidase inhibitor, and cytotoxic agent. Additional research highlights its antibacterial prowess against food-pathogenic bacteria, including Bacillus cereus, Escherichia coli, Staphylococcus aureus, and Salmonella typhimurium (Sitanggang *et al*., 2019; Anggraeni, 2020) [9, 2]. Given these attributes, the author is motivated to investigate the efficacy of 50% and 100% Andaliman Fruit (Zanthoxylum piperitum) extract in expediting wound healing post-tooth extraction in Wistar rats.

Research Methods

This experimental laboratory study adopts a randomized controlled design, employing a post-test-only control group design pattern. The research involves Wistar rats, 32 males aged 2-3 months and weighing 200-250 grams. All rats selected for the study are in good physical health.

The rats will be stratified into two distinct groups: the first group consisting of 16 rats treated with a 50% Andaliman Fruit (Zanthoxylum piperitum) extract, and the second group with 16 rats treated with a 100% Andaliman Fruit (Zanthoxylum piperitum) extract. The primary objective is to compare and evaluate the effectiveness of these two treatments in accelerating wound healing following tooth extraction.

To determine the sample size, the Federer formula was employed, which is expressed as $(t - 1)(r - 1) \geq 15$, where 't'

represents the number of treatments (2 treatments in this case), and 'r' represents the number of replications. Consequently, the minimum sample size for each treatment group was calculated to be 16 rats, ensuring statistical robustness and reliability in the study.

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

Tools

Tools used in research

1. Number-coded experimental animal cages.
2. Diagnostic set (mouth glass, sonde, tweezers).
3. Nierbeken.
4. Dental extraction forceps (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. Fruit Andaliman (Zanthoxylum piperitum) Extract 50%
2. Fruit Andaliman (Zanthoxylum piperitum) Extract 100%
3. Ketamine.
4. Formalin 10%.
5. Histology preparation material with Hematoxylin Eosin (HE) staining.
6. 70% alcohol as sterilization material.
7. 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 administering Fruit Andaliman (Zanthoxylum piperitum) 50% and Fruit Andaliman (Zanthoxylum piperitum) 100%. Collecting 3 kg of Fruit Andaliman (Zanthoxylum piperitum), the Fruit Andaliman (Zanthoxylum piperitum) was washed and divided into two parts to take the inner meat to obtain the gel. After washing, the flesh of the Fruit Andaliman (Zanthoxylum piperitum) was dried in an incubator at 500 ℃ for 72 hours. The dried flesh of the Fruit Andaliman (Zanthoxylum piperitum) was then pulverized using a blender until it became powder. Fruit Andaliman (Zanthoxylum piperitum) 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 Fruit Andaliman (Zanthoxylum piperitum) extract was then diluted with water to get 50% and 100% extract concentrations.

Before treatment, 32 rats were divided into 50% Zanthoxylum piperitum extract and 100% Zanthoxylum piperitum 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 Fruit Andaliman (Zanthoxylum piperitum) 50% in treatment group I and dropped Fruit Andaliman (Zanthoxylum piperitum) 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 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:

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 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 and Discussion

Data distribution and frequency of the number of fibroblast tissue per field of view in Wistar rats after tooth extraction in groups given 50% and 100% Andaliman Fruit extract (Zanthoxylum piperitum) can be seen as follows:

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

No	Number of Fibroblasts	Fruit Andaliman (Zanthoxylum piperitum)			
		Concentration 50%		Concentration 100%	
		n	%	n	%
1	No fibroblast tissue was found	0	0	0	0
2	A small number of fibroblasts (less than 10% per field of view)	7	44%	3	19%
3	Moderate amount of fibroblast tissue (10%-50% per field of view)	5	31%	5	31%
4	A large amount of fibroblast tissue (50%-100% per field of view).	4	25%	8	50%

Table 1 shows all samples found fibroblast tissue in administering Andaliman Fruit extract (Zanthoxylum piperitum) 50% and 100% post-extraction of Wistar Rat teeth. The number of fibroblasts found in the category was small (less than 10% per field of view) in the administration of Andaliman Fruit extract (Zanthoxylum piperitum) 50% after tooth extraction of Wistar Rats as many as 7 (44%) heads and in the administration of Andaliman Fruit extract (Zanthoxylum piperitum) 100% as many as 3 (19%) heads. The number of fibroblasts found in the medium category (10%-50% per field of view) in the administration of Andaliman Fruit extract (Zanthoxylum piperitum) 50% after tooth extraction of Wistar Rats as many as 5 (31%) heads and in the administration of Andaliman Fruit extract (Zanthoxylum piperitum) 100% as many as 5 (31%) heads. The number of fibroblasts found in the category is large (50%-100% per field of view) in the administration of Andaliman Fruit extract (Zanthoxylum piperitum) 50% after tooth extraction of Wistar Rats as many as 4 (25%) heads and in the administration of Andaliman Fruit extract (Zanthoxylum piperitum) 100% as much as 8 (50%) heads.

Table 2: Relationship between the number of tissue fibroblasts per field of view in Wistar rats after tooth extraction with the administration of Andaliman Fruit Extract (Zanthoxylum piperitum) at 50% and 100% concentrations.

Number of Fibroblasts	Fruit Andaliman (Zanthoxylum piperitum)		
	Concentration 50%	Concentration 100%	p
No fibroblast tissue was found	0	0	0,003*
Small number of fibroblasts (less than 10% per field of view) 3.	7	3	
Moderate amount of fibroblast tissue (10%-50% per field of view)	5	5	
A large amount of fibroblast tissue (50%-100% per field of view).	4	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 Andaliman Fruit Extract (Zanthoxylum piperitum) with a concentration of 50% and Andaliman Fruit Extract (Zanthoxylum piperitum) with a concentration of 100%, p = 0.003 (p <0.05).

Tooth extraction involves the removal of teeth, including both the complete tooth and any remaining roots, from the alveolar socket when the tooth is no longer treatable. This extraction process results in an injury, exposing the alveolar bone within the oral cavity. The wound incurred during tooth extraction represents anatomical damage or the partial destruction of tissue, primarily caused by trauma (Sorongan & Siagian, 2015). 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.

Rat tooth extraction will be performed 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. Andaliman Fruit Extract (Zanthoxylum piperitum) 50% was given to treatment group I. Andaliman Fruit Extract (Zanthoxylum piperitum) 100% to treatment group II shortly after tooth extraction as much as 0.05 ml daily by dropping. On the 5th day, the rat jaw was taken and fixed with 10% formalin for 24 hours at room temperature. The decalcification process used the ethylene diamine tetraacetic acid (EDTA 10%) solution at room temperature. The tissue was then dehydrated in a toluol alcohol solution (1:1) using the pure tool.

The fibroblast cell response was evaluated using Hematoxylin Eosin (HE) staining. Fibroblast density was assessed by counting the number of fibroblasts in 3 fields 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 researchers took the average day, namely on the fifth day (Stojanovic *et al*., 2011). 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 by giving 50% Andaliman Fruit Extract (Zanthoxylum piperitum) and 100% Andaliman Fruit Extract (Zanthoxylum piperitum), p = 0.003 (p <0.05).

This is seen in the distribution of data on the number of fibroblasts (50%-100% per field of view) in Fruit Andaliman (Zanthoxylum piperitum) 100% as many as eight samples and in Fruit Andaliman (Zanthoxylum piperitum) 50% only four pieces. The results of this study are supported by Shasti (2017), which states that Andaliman Fruit Extract, with a concentration of 8%, has the highest clear zone against the growth of S.aureus bacteria. In addition, the antibiotic effect of Andaliman Fruit extract at all concentrations was not significantly different, while cefotaxime with Andaliman Fruit extract at all concentrations had significant inhibition (Shasti, 2017). Andaliman fruit has the potential to be an antioxidant and glucosidase inhibitor. Andaliman is a spice widely used by the Batak community as a seasoning. Fruit andaliman has the potential to be an antioxidant and glucosidase inhibitor. The extract of andaliman fruit has the best antioxidant activity, with IC50 reaching 30.04 ppm. Fraction C (IC50 16 ppm) has acted as the most active glucosidase inhibitor and also contains flavonoid compounds of the around and flavanone group, which are the most active compounds as glucosidase inhibitors (Helmalia, 2019).

Anggraeni's research in 2020 provides insights into the composition of andaliman simple, revealing various characteristics and phytochemical components. According to the study, andaliman simplistic comprises 7.32% moisture content, 13.62% water-soluble juice content, 29.54% ethanol-soluble juice content, 4.100% total ash content, and 0.26% acid-insoluble ash content. Additionally, the phytochemical screening results indicate the presence of alkaloids, flavonoids, glycosides, saponins, tannins, and steroid/triterpenoid compounds in andaliman simple. These findings contribute to a comprehensive understanding of the chemical composition and potential therapeutic properties of andaliman (Anggraeni, 2020) [2]. Saragih's 2019 research highlights the therapeutic potential of andaliman seeds, emphasizing their rich content of active chemical compounds suitable for treatment. The identified active chemical compounds in andaliman seeds include phenolics, saponins, flavonoids, tannins, triterpenoids, and alkaloids. These secondary metabolite compounds exhibit diverse properties, such as antibacterial, antimicrobial, antiviral effects, and protein denaturation, effectively inhibiting bacterial growth during digestion. The knowledge of these active chemical compounds serves as a foundation for further exploration and utilization of andaliman seeds in developing remedies for various diseases (Saragih & Arsita, 2019). The study's findings suggest that the wound healing process is more effective with 100% Andaliman Fruit (Zanthoxylum piperitum) extract than a 50% concentration. This effectiveness is attributed to the higher concentration of active compounds in the 100% extract. The elevated content of these compounds contributes to an accelerated wound-healing process, indicating a positive correlation between the concentration of Andaliman Fruit extract and the speed of wound recovery.

Conclusion

Based on the results and discussions in this study, it can be concluded that andaliman Fruit Extract (Zanthoxylum piperitum) 50% and 100% effectively accelerate wound healing time after tooth extraction of Wistar rats.

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