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Effectiveness of the application of bawang dayak (eleutherine palmifolia l. merr) extracts on healing proccess in the osteitis alveolar post tooth extraction through fibroblast examination, collagen density and amount of osteogenesis (experimental study on sprague dawley mice)



Fajar Rezandaru,^{1*} Endang Syamsudin,¹ Indra Hadikrishna,¹ Vetnizah Juniantito²

Abstract

Objectives: The aim of this study was to analyze the potential for topical administration of Eleutherine palmifolia (l.) merr extract gel in the healing proccess of wound with osteitis alveolar as a complication after tooth extraction in Sprague dawley mice by observing fibroblast cell area, collagen density, and amount of osteogenesis.

Material and Methods: This study used a true experimental research method conducted on Sprague Dawley mice. Sample were divided into 3 groups, namely K1 (osteitis alveolar was not given any treatment), K2 (osteitis alveolar mice were given iodoform paste application every 3 days), and K3 (osteitis alveolar mice were given application of the extract gel Eleutherine palmifolia (I.) merr topically). On day 3, 5 and

10 necropsy was done and the tooth sockets in the form of soft tissue together with hard tissue was taken, then preparations were made and stained with masson trichrome to examined fibroblasts, collagen and osteogenesis.

Results: Topical application of the extract gel Eleutherine palmifolia (I.) merr showed the mean value of fibroblasts area and collagen density and the amount of osteogenesis were comparable to iodoform paste in alveolar osteitis healing.

Conclusion: this study is that the application of Eleutherine Palmifolia (I) merr extract gel has almost the same effectiveness as iodoform paste

Keywords: Alveolar osteitis, Eleutherine palmifolia I. merr, Fibroblast, Collagen, Osteogenesis

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Introduction

Post extraction complications can become serious and painful for patients and increase the number of patient visits to dental practice. Alveolar osteitis or dry socket is the most common complication occurring after tooth extraction and usually appears on days 3-5 after surgery.¹⁻³ The incidence is around 1-4% after the usual dental extraction procedure, and about 30% after the action of the lower third molar odontectomy.^{4,5} The clinical appearance shows an open alveolar, covered in necrotic tissue and accompanied by gingival inflammation. Histologically alveolar osteitis consists of remnants of blood clots and inflammatory cells such as neutrophils and lymphocytes around the socket. Some factors that can increase the incidence of osteitis are excessive trauma during extraction, bacterial infection, reduced blood supply from surrounding bone tissue due to vasoconstriction in local anesthetic drugs, use of contraceptive drugs or due to systemic conditions.6-8

The use of traditional medicines in healing wounds has been growing rapidly. This development is supported by the tendency of humans to treat naturally or return to nature. Dayak Onion (Eleutherine palmifolia (l.) Merr) is one of a well known plants among Dayak tribes who live on the island of Borneo. This Eleutherine palmifolia (l.) Merr originated from South America and has been used traditionally as a medicine in many parts of the world and as a spice in several Asian countries.9,10 The Dayaks used its roots to increase the production of breast milk, treat diabetes, breast cancer, stroke, hypertension and sexually transmitted diseases. In other areas this plants used to treat coronary heart disease, diuretics, emetics, laxatives, reduce prothrombin, antifertility and wound healing. In general, according to a study conducted by Song in 2009 stated that the roots of Eleutherine palmifolia (l.) Merr were efficacious as immunostimulants, anti-inflammatory, anti-tumor,

Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Padjadjaran University, Bandung, Indonesia

²Animal Teaching Hospital and Pathology Anatomy Laboratory, Faculty of Veterinary Medicine, Bogor Agricultural Institute, Bogor, Indonesia

*Correspondence to: Fajar Rezandaru, Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Padjadjaran University, Bandung, Indonesia farez_rezandaru@yahoo.com

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The aim of this study was to analyze the potential for topical administration of Eleutherine palmifolia (l.) merr extract gel in the healing proccess of wound with osteitis alveolar as a complication after tooth extraction in Sprague dawley mice by observing fibroblast cell area, collagen density and amount of osteogenesis.

Material and Methods

The research was conducted at the Chemistry Laboratory and Pharmacy Laboratory of the Bogor Agricultural Institute, Educational Animal Hospital of Bogor Agricultural University, Pathology Department, Clinical Department, Reproduction and Pathology, Faculty of Veterinary Medicine, Bogor Agricultural Institute. The time of the study was conducted on December 2018.

Samples were drawn randomly using Consecutive Random Sampling using the lottery model. Experimental animals that met the inclusion criteria were taken as research subjects. The research object was post tooth extraction wounds in twenty-seven Sprague-Dawley mice induced to develop alveolar osteitis (using 1: 1000 adrenaline vasoconstrictor and not given antibiotic therapy for 3 days after tooth extraction). Twenty-seven Sprague-Dawley mice were randomly divided into 3 groups. The first group was the osteitis (control) group treated with 1: 1000 adrenaline insertion for 1 minute on the left maxillary molars socket, the second group was the osteitis group given iodoform paste application every 3 days and the third group was the osteitis group given topical application Eleutherine palmifolia (l) merr extract gel dose 200 mg / kgBW / day.

On the 3rd, 5th and 10th day necropsy was performed and the extraction of dental sockets in the form of soft tissue together with hard tissue and 2 cc of blood collection. The tooth socket and tissue was soaked in 10% formalin solution for 24 hours, then decalcification was done using 20% HCl solution for 3-4 days. Then the paraffin block was made and cut with 4-5 µm size pieces. Preparations were made and stained with masson trichrome for examination of osteoblasts, collagen and osteogenesis. Assessment of the number of osteoblasts was calculated using software version J version 1.49n, Statistical analysis to compare the results of treatment using using non parametric statistical Kruskal Wallis and Mann Whitney tests. The scoring method used for collagen and osteogenesis level is ordinal type and is done by blind scoring according to Gibson-Corley scoring table 1 and table 2.14-16

Extraction of dayak onion (eleutherine palmifolia (l.) Merr) was carried out at the Pharmacy Laboratory of the Bogor Agricultural University (IPB), using the maceration method. The results of maceration are filtered using a Buchner funnel and vacuum. The extract obtained was then evaporated and concentrated with a low pressure evaporator at 65°C. The evaporation extract was dried in a vaporizer cup until thick extract was obtained. Gel of dayak onion made by using 8 grams of Na-CMC developed in 160 mL of warm distilled water, stirring until a clear gel formed. Then 40 grams of davak onion extract was put into the gel base, stirred until homogeneous. Oleum menthae added as much as 2g, then the distilled water is added to even 200 grams.

Results

On the results of day 3 negative control histopathology observation, there were still many sockets with necrotic tissue and neutrophil inflammatory cells surrounded by macrophage cells. Debris and blood clots with fibrin are found on the upper, edge and apical surfaces of the socket. The observation of negative control on day 5, the socket has begun to be filled with granulation tissue in the form of fibroblasts and collagen formed in several apical areas of the socket, but there are still found debris and inflammatory cells of neutrophils and macrophages, blood clots and also bacterial oral cavity in the surface and apical of the socket. On the 10th day histopathological control of negative controls, only a small amount of fibrosis and a little neovascularization were found figure 1A and figure 1B. Inflammatory cells and debris are still found on the surface, edges and apical sockets between the distribution of granulation tissue of fibroblast cells and their products, collagen. For some areas that are not deposited with debris, fibroblasts accumulate and little trabecular growth appears.

Observation of the treatment group iodoform paste application on day 3, showed a better histological picture, which are the dominance of fibroblast and collagen granulation tissue, new neovascularization and bone formation starting from the apical and edge of the socket. On day 5, the trabecular formation that starts from the apical and the edge has now begun to reach the center of the socket, although some alveolar space is still dominated by granulation tissue. Day 10 shows a lot of fibrosis and neovascularization and osteogenesis figure 2A and figure 2B. Some of the contents of the sockets are still dominated by granulation tissue of fibroblasts and collagen. The trabecula has formed at the apical edge of the socket toward the center and has almost reached the center of the socket.

Histopathological observations of the socket of the topical application group of Eleutherine palmifolia extract (1) for the third day, showed that part of the socket contents was still dominated by fibroblast and collagen granulation tissue. The trabecula has formed at the apical edge of the socket toward the center and has almost reached the center of the socket. On the 5th day, one of the sockets was filled with trabecula which almost covered the entire socket space. Day 10 shows a lot of fibrosis and osteogenesis figure 3A and figure 3B. Trabecula fills the edge of the socket towards the center and almost covers the entire socket space.

Percentage of fibtoblast and collagen and osteogenesis level shows at table 3, 4 and 5 below. In the control group, it was found that the number

 Table 1
 Scoring of distribution of fibroblasts / collagen with the gibson-corleyn system

Score	Description
0	There is absolutely no fibroblasts / collagen in wound tissue (0%)
1	The density of fibroblasts / collagen fibers is very low (1-10%) in wound tissue
2	Medium density fibroblasts / collagen fibers (> 10-30%) in wound tissue
3	Density of fibroblasts / tight collagen fibers (> 30 - 75%) in wound tissue
4	Density of fibroblasts / collagen fibers is very tight (> 75%) in wound tissue

Table 2	Scoring of	f osteoaenesis	distribution	with aibsor	n-corlevn system	

Score	Description
0	There is absolutely no osteogenesis (0%).
1	Very little (1-5%) osteogenesis in wound tissue
2	Moderate osteogenesis (> 5-20%) in wound tissue, especially at the periphery of wound tissue
3	Osteogenesis tends to be more (> 20 - 30%) at the periphery of the wound tissue
4	Dominant osteogenesis (> 30%) in wound tissue, especially in the peripheral area of the wound

Table 3 Percentage of fibroblasts in topical application of eleutherine palmifolia (I) merr extract gel with iodoform paste on days 3, 5 and 10

Variable	Control	lodoform paste	Eleutherine palmifolia (l.) merr extract	P value
Fibroblast day-3				
There are no fibroblasts (0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.100
Very low (1% -10%)	2 (66.7%)	0 (0.0%)	0 (0.0%)	
Medium (> 10% - 30%)	1 (33.3%)	2 (66.7%)	1 (33.3%)	
Many (> 30% -75%)	0 (0.0%)	1 (33.3%)	2 (66.7%)	
Very much (> 75%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Fibroblast day-5				
There are no fibroblasts (0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	$0.032^{(a)}$
Very low (1% -10%)	2 (66.7%)	0 (0.0%)	0 (0.0%)	
Medium (> 10% -30%)	1 (33.3%)	0 (0.0%)	0 (0.0%)	
Many (> 30% -75%)	0 (0.0%)	2 (66.7%)	0 (0.0%)	
Very much (> 75%)	0 (0.0%)	1 (33.3%)	3 (100.0%)	
Fibroblast day-10				
There are no fibroblasts (0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	$0.030^{(a)}$
Very low (1% -10%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Medium (> 10% -30%)	3 (100.0%)	0 (0.0%)	0 (0.0%)	
Many (> 30% -75%)	0 (0.0%)	1 (33.3%)	0 (0.0%)	
Very much (> 75%)	0 (0.0%)	2 (66.7%)	3 (100.0%)	

Description: Different superscript letters on the same line show significant differences (p < 0.05)

			Eleutherine palmifolia	
Variable	Control	lodoform paste	(I.) merr extract	P value
Collagen day 3				
There is no collagen (0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.100
Very low (1% -10%	2 (66.7%)	0 (0.0%)	0 (0.0%)	
Medium (> 10% - 30%)	1 (33.3%)	2 (66.7%)	1 (33.3%)	
Tight (> 30% -75%)	0 (0.0%)	1 (33.3%)	2 (66.7%)	
Very tight (> 75%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Collagen day 5				
There is no collagen (0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.032 ^(a)
Very low (1% -10%	2 (66.7%)	0 (0.0%)	0 (0.0%)	
Medium (> 10% - 30%)	1 (33.3%)	0 (0.0%)	0 (0.0%)	
Tight (> 30% -75%)	0 (0.0%)	2 (66.7%)	0 (0.0%)	
Very tight (> 75%)	0 (0.0%)	1 (33.3%)	3 (100.0%)	
Collagen day 10				
There is no collagen (0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.030 ^(a)
Very low (1% -10%	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Medium (> 10% - 30%)	3 (100.0%)	0 (0.0%)	0 (0.0%)	
Tight (> 30% -75%)	0 (0.0%)	1 (33.3%)	0 (0.0%)	
Very tight (> 75%)	0 (0.0%)	2 (66.7%)	3 (100.0%)	

Table 4Percentage of collagen density in topical application of eleutherine palmifolia (I)merr extract gel with iodoform paste on days 3, 5 and 10

Description: Different superscript letters on the same line show significant differences (p<0.05)

Table 5Percentage of osteogenesis in topical application of Eleutherine palmifolia (l.) Merr
extract gel with iodoform paste on days 3, 5 and 10

			Eleutherine palmifolia	
Variable	Control	lodoform paste	(I.) merr extract	P value
Osteogenesis day 3				
No osteogenesis	3 (100.0%)	0 (0.0%)	0 (0.0%)	$0.029^{(a)}$
In a very small amount (1%-5%)	0 (0.0%)	1 (33.3%)	3 (100.0%)	
Moderate (>5%-20%)	0 (0.0%)	2 (66.7%)	0 (0.0%)	
Tend to be more (>20%-30%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Dominant (>30%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Osteogenesis day 5				
No osteogenesis	3 (100.0%)	0 (0.0%)	0 (0.0%)	$0.023^{(a)}$
In a very small amount (1%-5%)	0 (0.0%)	1 (33.3%)	0 (0.0%)	
Moderate (>5%-20%)	0 (0.0%)	2 (66.7%	0 (0.0%)	
Tend to be more (>20%-30%)	0 (0.0%)	0 (0.0%)	2 (66.7%)	
Dominant (>30%)	0 (0.0%)	0 (0.0%)	1 (33.3%)	
Osteogenesis day 3		10		
No osteogenesis	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.029 ^(a)
In a very small amount (1%-5%)	3 (100.0%)	0 (0.0%)	0 (0.0%)	
Moderate (>5%-20%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Tend to be more (>20%-30%)	0 (0.0%)	2 (66.7%)	0 (0.0%)	
Dominant (>30%)	0 (0.0%)	1 (33.3%)	3 (100.0%)	

Description: Different superscript letters on the same line show significant differences (p <0.05)







Figure 2 Fibroblasts (purplish red), collagen density and osteogenesis (blue).a) 10x objective enlargement, b) 40x objective enlargement in the 10th day iodoform paste group staining Masson Trichrome. There is a lot of fibrosis (red arrow) and a lot of neovascularization (black arrow)



Figure 3 Fibroblasts (purplish red), collagen density and osteogenesis (blue).
 A. 10x objective enlargement, B. 40x objective enlargement in the Eleutherine palmifolia extract gel group (l.) on the 10th day of staining Masson Trichrome. Looks a lot of fibrosis (red arrow) and lots of osteogenesis (black arrows)

of fibroblasts with very little category and in the idioform paste group found a number of fibroblasts with many categories. On the 10th day, the group using Eleutherine palmifolia (l) merr extract had a very large number of fibroblasts. Whereas in the control group the number of fibroblasts with the moderate category and in the idioform paste group had a large number of fibroblasts with a large category. Based on the results of the research data, a statistical test of different tests was conducted to determine whether there were differences between the control group and the treatment of the application of iodoform paste and gel extract of Eleutherine palmifolia (l) merr. Because the distribution is not normal and is ordinal in scale, the hypotheses of the Kruskal Wallis, Wilcoxon / Mann Whitney non-parametric statistics was performed.

The results of the calculation of Mann Whitney test showed that in general the control group was significantly different from idioform and dayak onions (p <0.05), but dayak onions had almost the same effectiveness as idioform paste (p> 0.05).

Discussion

In this study, the iodoform treatment group and eleutherine palmifolia (l. Merr) topical gel showed that the average area of fibroblasts was significantly higher than the control group observed at days 3, 5 and 10. This was caused by cell activity - Increased fibroblast cells after application of iodoform and topical paste of extract gel eleutherine palmifolia (l. merr).¹⁵

Eleutherine Palmifolia (l.) Merr contain of various phytochemical compounds such as flavonoids which are anti-inflammatory, antioxidant, and analgesic, alkaloids are antibacterial, and saponins that are useful in influencing collagen in the early stages of tissue repair. Eleutherine Palmifolia (l.) Merr as a prohealing agent has wound healing activities obtained from phytochemical substances. The potential of iodoform as a treatment for alveolar osteitis has also been clinically proven and declared effective for healing secondary wounds and preventing premature wound closure which causes reaccumulation of bacteria.¹⁵

Eleutherine species contained of naptoquinone which have antiseptic, antibacterial, analgesic and anti-inflammatory effects so that they can accelerate wound healing. Tannin and flavonoids function to stimulate fibroblasts and collagen formation so that they are effective for wound care. In addition, the saponin content can stimulate differentiation of osteoblasts to accelerate bone healing.¹⁶

This study revealed that topical administration of Eleutherine Palmifolia (L.) merr extract gel in alveolar osteitis proved to have a positive effect on the increase of osteogenesis which was comparable to iodoform paste. This means that Eleutherine Palmifolia (l) merr has the potential to cure alveolar osteitis which is comparable to iodoform paste. Meanwhile, if the average area of fibroblasts and collagen does not produce a statistically higher value than the control, this is because the acceleration of wound healing has occurred earlier in the treatment group than the control group.^{16,17}

Conclusion

The general conclusion of this study is that the application of Eleutherine Palmifolia (l) merr extract gel has almost the same effectiveness as iodoform paste on days 3 and 5 on examination of fibroblasts and collagen, but has better effectiveness on osteogenesis examination on the 10th day.

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Conflict of Interest

The authors report no conflict of interest.

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