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Effects of 1% and 3% Mobe Leaf Extract Gel on Socket Wound Healing after Tooth Extraction

(Efek Gel Ekstrak Daun Mobe 1% dan 3% pada Penyembuhan Luka Soket Pasca Pencabutan Gigi)

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Abstract

Tooth extraction is a process of removing teeth from the alveolar bone. In wound healing, fibroblast are very important cells. The main purpose of this study was to determine the effect of mobe leaf 1% and 3% extract gel (*Artocarpus lakoocha*) on fibroblast proliferation in post extraction tooth socket wound healing. This research used 16 samples of wistar rats, divided into 4 groups, a positive control group, a negative control group and a 1% and 3% mobe leaf extract gel group. The left mandibular incisors were extracted, then 1% and 3% gels of mobe leaf extract were applied on day 1 to day 7. Data analysis was calculated using the Kruskal-Wallis test on clinical data and one way ANOVA test for microscopic. The result of the socket wound healing activity test for a good concentration of mobe leaf extract gel was 3%. This research shows significant results with p-value of 0.018 (< 0.05) on the closure of the socket wound clinically which means the closure of the wound accelerates because of the mobe leaf 3% extract gel treatment. The distance of fibroblast on microscopically shows significant results with a p-value of 0.002 (< 0.05), which means that there was an enlargement of the distance fibroblast at the socket wound closure with application of mobe leaf 3% extract gel. From the results of the study it can be concluded that mobe leaf 3% extract gel has the best ability to show acceleration the closure of the socket wound either clinically or microscopically.

Key words: mobe leaf; tooth extraction; wound healing; fibroblast

Abstrak

Pencabutan gigi merupakan suatu proses mengeluarkan gigi dari tulang alveolar. Sel fibroblas merupakan sel yang sangat penting dalam penyembuhan luka. Tujuan dari penelitian ini adalah untuk mengetahui efek gel ekstrak daun mobe (*Artocarpus lakoocha*) 1% dan 3% terhadap proliferasi fibroblas pada penyembuhan luka soket pasca pencabutan gigi. Penelitian ini menggunakan 16 ekor sampel tikus putih galur wistar, dibagi atas 4 kelompok, kelompok kontrol positif, kelompok kontrol negatif dan kelompok gel ekstrak daun mobe 1% dan 3%. Gigi insisivus kiri rahang bawah dicabut, kemudian diaplikasikan gel ekstrak daun mobe 1% dan 3% pada hari ke-1 sampai hari ke-7. Analisis dengan metode Kruskal-Wallis untuk penutupan luka secara klinis dan one way ANOVA untuk jarak fibroblas secara mikroskopis. Hasil dari uji aktivitas penyembuhan luka soket dengan sediaan gel ekstrak daun mobe konsentrasi yang baik adalah 3%. Penelitian ini menujukkan hasil yang signifikan dengan p-value sebesar 0,018 (< 0,05) pada penutupan luka secara klinis, yang berarti terdapat percepatan penutupan luka soket dengan perlakuan gel ekstrak daun mobe 3%. Hasil yang siginifikan didapati pada jarak firoblas secara mikroskopis dengan p-value sebesar 0,002 (< 0,05) yang berarti terdapat perbesaran jarak fibroblas dengan perlakuan gel ekstrak daun mobe 3%. Kesimpulan dari hasil penelitian ini adalah gel ekstrak daun mobe 3% memiliki kemampuan paling baik yang menunjukkan percepatan penutupan luka baik secara klinis maupun secara mikroskopis.

Kata kunci: daun mobe; pencabutan gigi; penyembuhan luka; fibroblast

INTRODUCTION

Tooth loos has become a global public health concern of immense proportion.[1] In Indonesia, oral healthcare for tooth extraction is very high at 79.6%.[2] Tooth extraction is a process of removing a tooth from the alveolar process. [3] Post-tooth extraction sockets cause discomfort in the oral cavity, such as pain and can interfere with eating. For this reason, the wound healing process is something dentists need to pay attention to.[4]

dergoes a healing process consisting of soft and hard tissues healing. The soft tissue that undergoes healing are the gingival connective tissue and the gingival epi-thelium, while the hard tissue that undergoes a healing process is the alveolar bone tissue.[4]

The healing process for socket wounds in both soft and hard tissues begins with the formation of a blood clot in the tooth socket. The blood clot will develop into granulation tissue that contains blood vessels, fibroblasts and inflammatory cells. Epithelium will cover the surface of the granulation tissue, debris and bone fragments. Granulation tissue will develop into connective tissue that covers the surface of the bone so that the bone in the tooth socket wall is able to regenerate.[4]

Fibroblasts are very important cells in the early stages of wound healing and begin to form during the proliferative stage from the third day after injury. Between day 3 and day 5, fibroblasts begin to migrate to the wound site and produce a predominant number of fibroblasts at the wound site. Fibroblasts act to break down blood clots, forming Extracellular Matrix (ECM) and collagen fibres to support the formation of new bone effectively in socket.[5]

The World Health Organization (WHO) reports that around 80% of the world depends on traditional herbal medicines for primary health care. One of the plants is the mobe plant. Mobe or Artocarpus lakoocha is a member of the Moraceae family, this plant is known as Monkey Jack in English.[6,7]

Mobe leaves contain flavonoids, tannins, saponins and glycosides. Flavonoids and tannins are antiseptics that play an important role in protecting wounds from bacterial growth in the inflammatory phase and can help accelerate wound healing and increasing the number of capillary blood vessel formation and fibroblast cells. The main component of mobe leaf is found in flavonoids such as artocarpin. Artocarpin can induce an early inflammatory phase by increasing the production of TGF-β so as to accelerate the proliferation and migration processes of fibroblasts, increasing collagen deposition and thus the wound healing process takes place quickly.[8]

Based on this background, the researchers were interested in seeing the effect of 1% and 3% of mobe leaf extract gel on accelerating wound healing both clinically by closing the wound closure distance and microscopically by enlarging the fibroblast migration distance on socket wound healing after tooth extraction of Wistar strain white rats.

MATERIALS AND METHODS

This study has received approval from the Animal Research Ethics Commission (KEPH) at the Faculty of Mathematics and Sciences, University of North Sumatera (Animal Research Ethics Committees/ AREC), number: 00115/KEPH-FMIPA/2020. The used mobe leaves come from the Laguboti District, Toba Samosir, North Sumatera Province. The samples used were male Wistar rats with an average body weight of 150-250 grams and healthy which was characterized by active movement, clean hair, clear eyes, and had never received treatment before. The number of samples was 16, divided into 4 groups, the negative control group was not given gel application, the positive control group was given Gengigel®, the control group 3 was given 1% mobe leaf extract gel application, and the control group 4 was given 3% mobe leaf extract gel application and was observed on the seventh day.

This mobe leaf is made using the maceration technique. Mobe leaves are washed, then weighed as much as 200 grams and put in a dark bottle, then add 200ml of 96% ethanol solvent. Then soak for the first 6 hours while stirring several times, then let it still for 18 hours. Then separate the macerate by filtering it. Repeat the filtering process at least twice with the same type and amount of solvent. Next, collect all the macerate, then evaporate it with a rotary evaporator until a thick extract is obtained. Making a gel base was done using *hydroxypropyl methylcellulose* and carbopol, which stirred homogeneously, then the extract was added as much as 0.4 grams for 1% and 1.2 grams for 3%.

The white rats were acclimatized beforehand for 1 week. Anaesthesia was done using ketamine and xylazine at a dose of 25 mg/kg and 10 mg/kg, by injection in the intraperitoneal, and then the left mandibular incisor was removed using clamp arteries with a rotational motion, until the tooth was completely extracted. After extraction, irrigate the socket with aquadest to remove debris after tooth extraction.

Apply Gengigel® and mobe leaf extract gel to the socket wound using a 0.1ml syringe, directly to the wound after occurrence of the wound which is counted as day 1 twice a day, namely morning and evening. Wound healing was observed clinically by closure/ shrinkage of the socket wound on the seventh day, then the mice were sacrificed on the seventh day after tooth extraction and histological preparations was made and examined by Haematoxylin Eosin to observe the large migration distance of fibroblasts.

The process of making histological preparations in fixation, dehydration, clearing, embedding, blocking sectioning, staining and mounting. To determine migration distance of fibroblast cells, staining was done using Haematoxylin Eosin. The preparations were observed under a light microscope with a magnification of up to 10x to see migration distance of fibroblasts. Data analysis was done using One-Way Anova statistical test.

RESULTS

Clinical and microscopic wound closure data in this study were first tested by normality test and found that clinical wound closure variable group data were not normally distributed and the fibroblast distance variable data were normally distributed.

Assessment of the mean measurement of socket closure distance after tooth extraction with the Kruskal Wallis test are shown in Table 1. The data obtained in this study showed that the significant value was $p < 0.05$ ($p = 0.018$), so there was a significant difference in clinical wound closure between group. In the control group 4, 3% mobe leaf extract gel had the best clinical results for socket wound closure after tooth extraction of rats.

Based on table 1, the best results of clinical socket wound closure post-tooth extraction are found in the 3% mobe leaf extract gel group namely 1.80 ± 0.14mm. it can be concluded that 3% mobe leaf extract gel application can help accelerate the process of socket wound closure after tooth extraction in rat because it has the best increased acceleration in socket wound closure.

Furthermore, the Kruskal Wallis test was carried out and the result was 0.018 which indicated a significant difference between groups. Then the Man-Whitney test was carried out to determine any significant differences between each group.

Based on the results of the Man-Whitney test, there was a clinically significant difference in wound closure between the 3% mobe leaf extract gel and 1% mobe leaf extract gel group, the 3% mobe leaf extract gel group with negative control, the 1% mobe leaf extract gel group with negative control. negative group, negative control group with positive control with a p-value of <0.05.

Assessment of the mean measurement of fibroblast distance in the post extraction socket with One-Way Anova test are shown in table 3. The data obtained in this study showed that the significant value was $p < 0.05$ ($p = 0.002$), so there was a significant difference in clinical wound closure between group. In the control group 4, 3% mobe leaf extract gel had the best clinical results for socket wound closure after tooth extraction of rats.

Based on table 3, it is obtained that the best average fibroblast distance in post-tooth extraction socket wounds is found in the 3% mobe leaf extract gel group of 107.68 ± 21.77 µm, thus it can be concluded that applying the 3% mobe leaf extract gel can help accelerate the process of socket wound closure after tooth extraction of rat.

Furthermore, the One-Way Anova test was carried out and the results were 0.002 which indicated a significant difference between groups. Followed by the LSD test and obtained significant results between the 3% mobe leaf extract gel treatment group and 1% mobe leaf extract gel, the 3% mobe leaf extract gel group and negative control, the 1% mobe leaf extract gel group and the negative group, negative control group and positive control with p-value of <0.05.

DISCUSSION

Based on this study, there was a significant difference in the group with 3% mobe leaf extract gel which shows fastest post-tooth extraction socket wound closure compared to the untreated group, both clinically and microscopically by observing mean distance of fibroblasts with haematoxylin eosin staining which is the standard for tissue histological examination. [9]

This can happen because the leaves of mobe have secondary metabolites such as flavonoids, tannins, saponins. Flavonoids play a role in tissue proliferation and remodelling phases, namely by increasing vascularization so that the supply of oxygen and nutrients to injured tissues and cells are maximized, increasing collagen synthesis so as to accelerate the wound healing process. Tannins accelerate the wound healing process through various cellular mechanisms, namely reducing reactive oxygen and free radicals, increasing wound closure by producing fibroblasts and increasing the formation of blood capillaries and by being antimicrobial which increase epithelialization. Saponins play a role in stimulating VEGF (Vascular Endothelial Growth Factor) and increases the number of macrophages that migrate to the wound area. [10,11,12]

In addition to the secondary metabolites above, artocarpin is also present in mobe leaves extract. Artocarpin is a compound that is prenylated from flavonoids which are found in many types of artocarpus plants. These secondary metabolites have anti-inflammatory activity. In a study by Daud stated that artocarpin exhibits anti-inflammatory activity by inhibiting free radical production, chemotaxis of cell phagocytes and reduction of oxidative stress. And another study by Ju Yeh shows that artocarpin can induce an earlier inflammatory phase by increasing the production of TGF-β, increasing the proliferation and migration of fibroblasts, increasing collagen deposition and thus the wound healing takes place quickly. [8,13] The results of this study are in line with research conducted by Adriani in 2019 which showed that 3% mobe leaf extract influenced the acceleration of wound closure compared to 1% mobe leaf extract and the untreated group. Then another study conducted by Nesa in 2015, showed that mobe leaf extract can be used as an alternative or additional herbal medicine for anti-inflammatory treatment because of its flavonoid and saponin compounds. In addition, research conducted by Bhattacharya in 2019 showed that mobe leaf extract has a strong antioxidant effect found in tannins by inhibiting free radicals so that wound healing takes place quickly. Mobe leaves also have antimicrobial activity which is able to fight a number of bacterial species. [10,14,15]

In conclusion, 3% Mobe leaf extract gel can accelerate the healing process of socket wounds after tooth extraction of Wistar rats, both clinically and microscopically on the seventh day.

TABLES

Table 1. Mean of clinical wound closure

Variabels	N	Mean ±SD	*P-Value*
Positive Control	4	1.70±0.14	
Negative Control	4	1.37±0.05	
Mobe leaf 1%	4	1.60±0.14	0.05
Mobe leaf 3%	4	1.80±0.14	

Table 2. Man-Whitney test

Variabels	N	Mobe Leaf Extract Gel 3%	Mobe Leaf Extract Gel 1%	Negative Control
Mobe leaf 3%	4	-		
Mobe leaf 1%	4	0.016	-	
Negative Control	4	0.017	0.044	-
Positive Control	4	0.234	0.234	0.017

Table 3. Mean of microscopic wound closure on the 7th day

Variabels	N	Mean ± SD	*P-Value*
Positive Control	4	100.46±5.01	
Negative Control	4	61.39±12.11	
Mobe leaf 1%	4	89.12±6.75	0.05
Mobe leaf 3%	4	107.68±21.77	

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