

Research Report

The effect of mengkudu gel (*Morinda citrifolia* Linn.) in accelerating the escalation of fibroblast post extraction

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ABSTRACT

Background: Tooth extraction is one of treatment frequently done by dentists in clinics, hospital, and even private practices. One thing that is needed to be observed after the treatment is the speed of wound recovery process. Mengkudu is commonly used as medicinal treatments, some of them to heal wounds, but there had never been research of the use of mengkudu fruit in wound recovery after tooth extraction. **Purpose:** The aim of this study was to investigate the effect of mengkudu gel in accelerating the escalation of fibroblast post tooth extraction on Dawley rats. **Methods:** This study was used post test only control group design. Thirty male Dawley rats weigh between 250–300 grams, 3 months of age are being used. Tooth extraction is being done on lower left incisor. The 30 rats are divided into three groups. The data were analyzed statistically using One-Way ANOVA and $LSD_{0.05}$. **Result:** The result of every tested group showed $p > 0.05$, therefore all the data had a normal distribution. Therefore, a One-Way ANOVA test with 5% significant rate was done and continued by LSD test to find a significant difference in each groups. Examination showed there was significant difference in fibroblast amount between the group with mengkudu gel and two other groups ($p < 0.05$). **Conclusion:** The application of mengkudu gel can accelerate the escalation of fibroblast after the tooth extraction on Dawley rats.

Key words: fibroblast, mengkudu, extraction

ABSTRAK

Latar Belakang: Ekstraksi gigi merupakan perawatan yang sering dilakukan oleh dokter gigi baik di klinik, rumah sakit, dan praktek pribadi. Satu hal yang perlu diperhatikan setelah pencabutan gigi adalah kecepatan penyembuhan luka bekas cabut. Mengkudu merupakan bahan yang sering digunakan untuk pengobatan, salah satu diantaranya adalah untuk penyembuhan luka, namun penelitian untuk kesembuhan luka pasca cabut gigi yang menggunakan mengkudu belum pernah dilakukan sebelumnya. **Tujuan:** Tujuan penelitian ini untuk mengetahui efek gel mengkudu dalam mempercepat peningkatan jumlah fibroblas setelah pencabutan gigi tikus Dawley. **Metode:** Penelitian ini menggunakan rancangan penelitian post test only control group design. 30 ekor tikus Dawley jantan, berat 250–300 gram, usia 3 bulan. Gigi yang dicabut dilakukan pada insisif rahang bawah. 30 ekor tikus Dawley dibagi dalam 3 kelompok penelitian. Data sampel yang didapat dianalisa menggunakan One-Way ANOVA dan $LSD_{0.05}$. **Hasil:** Hasil dari masing-masing kelompok adalah $p > 0.05$, yang menunjukkan bahwa seluruh data berdistribusi normal. Tes One-Way ANOVA dengan taraf kemaknaan 5% digunakan kemudian dilanjutkan dengan tes LSD untuk mengetahui perbedaan pada masing-masing kelompok perlakuan. Hasil perhitungan menunjukkan ada perbedaan bermakna pada jumlah fibroblas antara gel mengkudu dengan 2 kelompok lain ($p < 0.05$). **Kesimpulan:** Aplikasi gel mengkudu dapat mempercepat peningkatan jumlah fibroblast setelah pencabutan gigi pada tikus Dawley.

Kata kunci: fibroblas, mengkudu, pencabutan gigi

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INTRODUCTION

Indonesia is one of the tropical countries which is rich in many different kinds of plants that useful for health. One of the kinds of plants which is known for its many usefulness for health is mengkudu (*Morinda citrifolia* Linn.).^{1,2}

Mengkudu fruit has a complex chemical contents, some of them are: *Antraquinone*, *Xeronine*, *Proxeronine*, sorbic acid, terpenoid compound, anti bacterial substance, *Scopoletin*, and *Damnacanthal*. This substance acts as antimicrobial, especially bacteria, fungus, and it also has an important role in taking care of inflammation and allergy, as well as one of the important nutrition which is needed for wound recovery.³

Mengkudu assists in the availability of xeronine in body. This enzyme is crucially needed in the every metabolism activity of the human body. That is why this fruit can help in strengthening body immune system and useful as adaptogen, to balance the work of cell in human body. The enzyme exists in mengkudu in the *proxeronine* compound.²

Wound recovery process from tooth extracted sometimes have an experiences disturbance so that complication may happen. Some researchers claim that the use of medicine post tooth extraction can reduce the possibility of complication and it's often expected to be able to accelerate blood coagulation process, so that it will also accelerate the process of wound recovery.⁴

Healing or recovery of damaged tissue basically is a replacement of the damaged tissue with new normal tissue. The process of tissue recovery is the first stage of dynamic processes.⁵ The healing process is important for normal structure maintenance, function, and life perpetuity of an individual, one of which has important role is fibroblast. Fibroblast synthesize collagen, elastin, glycoaminoglycans, proteoglycans and multiadhesive glycoproteins. Fibroblast are the most common cells in connective tissue and are responsible for the synthesis of extracellular component such as collagen fiber. Collagens constitute the most abundant proteins found in the body. All collagens are composed of three polypeptide alpha chains coiled around each other to form the typical collagen triple helix configuration. Common features include the presence of the amino acid glycine in every third position, a high proportion of proline residues. Hydroxylation of proline and lysine occurs after these amino acids are incorporated into polypeptide chains, hydroxylation begins after the peptide chain has reached a certain minimum length and is still bound to ribosomes. The two enzymes involved are peptidyl proline hydroxylase and peptidyl lysine hydroxylase.⁴

Cells with intense synthetic activity are morphologically distinct from the quiescent fibroblasts that are scattered within the matrix they have already synthesized. The quiescent fibroblast is smaller and tends to be spindle shaped, it has fewer processes, a smaller, darker, elongated nucleus.^{6,7} Fibroblast, particularly those activated and responding to some type of stimulation, such as

inflammation or mechanical forces, secrete a number of growth factor, cytokines, and inflammatory mediators. The repertoire of factors varies depending on the location and type of fibroblast but may include interleukin-1, interleukin-6, interleukin-8, tumor necrosis factor α , prostaglandin E2, platelet-derived growth factor, insulin-like growth factor, transforming growth factor β , vascular endothelial growth factor, basic fibroblast growth factor, hepatocyte growth factor, and keratinocyte growth factor.

In response of damage tissue, fibroblast proliferates and actively synthesizes matrix components and upon more specific observation on cellular level, the active fibroblast has an abundant and irregularly branched cytoplasm and also appears bigger and more basophilic. Its nucleus is ovoid, large, and pale staining, with fine chromatin and a prominent nucleolus. The cytoplasm is rich in rough endoplasmic reticulum, and the golgi complex is well developed. Fibroblast starts to appear on the wounded area three days after the laceration happens.⁶ Wound is a damage on body tissue which is caused by several kinds of factors. Wound recovery is an attempt to fix the damage. The main component in wound recovery process is fibroblast. Fibroblast is the cell which responsible for collagen synthesis. Fibroblast is a cell which comes from a mesenchymal tissue which is also embryonic tissue for connective tissue, bone tissue, cartilage, etc. fibroblast produce extracellular component from growing connective tissue. Fibroblast exist in all fibrous connective tissue in the body and responsible to synthesize precursors from collagen, reticular and elastic fibres.⁸

Mengkudu is commonly used as medicinal treatments, some of them to heal wounds, but there had never been research of the use of mengkudu fruit in wound recovery after tooth extraction. The purpose of this research is to know the effect of mengkudu gel in accelerating the escalation of fibroblast amount post tooth extraction on Dawley rats.

MATERIALS AND METHODS

This study is an experimental laboratory research using the post-test only control group design. Thirty male Dawley rats weigh between 250–300 grams, 3 months of age are being used. Have well condition, food and drink water given ad libitum. This animal is used because tooth extraction on Dawley rats is easier with sufficiently wide socket extraction wound for applying mengkudu gel. Tooth extraction is being done on lower left incisor. The choosing of lower incisor is based on the structure and anatomical form of Rat's teeth which enable extraction to be done. The 30 rats are divided into three groups. On the first group, after the extraction is done, mengkudu gel is applied on the extraction wound. On the second group, povidone iodine is applied on the extraction wound, and on the third group, sterile aquades is applied on extraction wound.

Mengkudu fruit which is made into gel will be easier to be put into the extraction wound socket because of its solid, soft and elastic characteristics. This gel forms makes the substance durable in extraction wound socket, so that it helps the body in wound recovery process.

The making of mengkudu fruit gel is uses the mixture of CMC Na and distillation of mengkudu fruit. CMC Na is derivative of cellulose and often being uses in food industry.⁹ The characteristics of CMC Na are for thickening, stabilizer, gel maker and in some things as emulsifiers. In hydrocolloid emulsion system it doesn't function as emulsifiers, but more as substance which gives stabilization. This CMC Na is easily soluble in hot or cold water, so it is easy to use. It is used as stabilizer because it's easily obtainable and reasonably priced.¹⁰

After three days, all animal was decapitation and the socket post extraction preserved on the slide. Buffered isotonic solution of 4% formaldehyde was used for fixatives. 70% to 100% ethanol was used to extracted the water from the fragment. The ethanol then replaced with a solvent miscible with the embedding medium. In paraffin embedding, the solvent used is xylene. Once the tissue is impregnated with the solvent, it is placed in melted paraffin in the oven at 58–60° C. The heat causes the solvent to evaporate, and the space within the tissue become filled with paraffin. The tissue together within its impregnating paraffin hardens taken out of the oven. Tissues embedded with plastic resin dehydrated in ethanol. The hard blocks containing the tissues are then taken to a microtome, and sliced into thin sections 1–10 µm. The sections are floated on water and transferred to glass slide to be stained. Staining tissue with Masson Trichrome was done to make the various tissue component conspicuous. Under the light microscope, tissue are examined via a light beam that is transmitted through the tissue using image magnified 400 times.⁴

Every tested group data were analyzed by one-way ANOVA test with 5% significant rate and continued by LSD test if there was significant difference.

RESULT

The mean and standard deviation of the amount of fibroblast post extraction on Dawley rats is shown in the Table 1. A Kolmogorof-Smirnov test was carried out on the data to determine the normality of distribution. The result of every tested group showed $p > 0.05$, therefore all the data had a normal distribution. Therefore, a One-Way ANOVA test with 5% significant rate was done and continued by LSD test if there was a significant difference. The result on the third day examined via a light beam that is transmitted through the tissue using image magnified 400 times shows that the amount of fibroblast on the group which is given mengkudu gel is much more than the control group and the povidone iodine given group. Table 2 shows there is significant difference in each group treatment $p < 0.05$ ($p = 0.001$). After that, the data was continued with LSD test.

Table 1. Mean amount of fibroblast and standard deviation in treatment group

Group	Mean	SD
Mengkudu gel	49.80	3.19
Povidone Iodine	39.70	1.33
control	41.10	1.85

Table 2. The ANOVA result from fibroblast cell in treatment group

Variation source	Free level	Mean Square	F. account	Probability
Between Group	2	299.433	58.331	0.001
In Group	27	5.133		
Total	29			

Post-Hoc test showed there is no significant difference in fibroblast amount between control group and the povidone iodine given group but the comparison between mengkudu gel given group with two other groups shows the significant difference in fibroblast growth (Table 3).

Table 3. The result of LSD test shows the difference in fibroblast amount comparison between mengkudu gel with two other groups

	Mengkudu	Povidone Iodine	Control
Mengkudu			
Povidone Iodine	S		
Control	S	NS	

DISCUSSION

After an injury to either oral mucosa or socket post extraction, blood clot is formed in the area and the inflammatory response is triggered by its white blood cells. If the source of injury is removed, tissue repair can begin within the next few days. The epithelial cells at the periphery of the injury will lose their desmosomal intercellular junction and migrate to form a new epithelial surface layer beneath the clot. It is very important in repairing the connective tissue and must be retained in the first day of repairing because it acts as a guide to form a new surface. After the epithelial surface is repaired, the clot is broken down by enzymes because it is no longer needed. Repair of the epithelium is tied to the repair taking place in the deeper connective tissue.¹¹

Fibroblast synthesize proteins, such as collagen and elastin, that form collagen, reticular and elastic fibers. They also involved in the production of growth factors that influence cell growth and differentiation.⁴

The significant escalation of fibroblast amount on the use of mengkudu is caused by the existence of substance in mengkudu, one of them is Anthraquinone. Anthraquinone is the main component in mengkudu, the

antibacterial characteristic from Anthraquinone helps body to avoid infection, fever and all bacterial-related disease. Anthraquinone increases the biosynthesis regulation from type I collagen, polypeptide α chains are assembled on polyribosomes bound to rough endoplasmic reticulum membranes and injected into the cisternae as procollagen molecules. Collagens constitute the most abundant proteins found in the body, that plays important role in wound healing process.

This study showed that fibroblast is more active, so it has an abundant and irregularly branched cytoplasm and also appears bigger and more basophilic. Its nucleus become ovoid, large, and pale staining, with fine chromatin and a prominent nucleolus. It is also participate in the remodeling of connective tissue through the degradation of collagen and other matrix molecules and their replacement by newly synthesized molecules. Two mechanisms for the degradation of collagen which is the secretion by cells of enzymes that sequentially degrade collagen and other matrix molecules extracellularly and the selective ingestion of collagen fibrils by fibroblast and their intracellular degradation.¹² The collagen triple helix is highly resistant to proteolytic attack. The matrix metalloproteinase family is a large family of proteolytic enzymes that includes collagenases (MMP-1, MMP-8 and MMP-13), gelatinases (MMP-2 and MMP-9), metalloelastase (MMP-12), stromelysin (MMP-7). These enzyme are capable of degrading collagen and other matrix macromolecules into small peptides extracellularly. The matrix metalloproteinases are synthesized and secreted by fibroblast, inflammatory cells and some epithelial cells. Extracellular degradation often occurs in inflammatory lesions or when large amount of collagen must be degraded rapidly. The MMPs are secreted as in active precursor and must be cleaved proteolytically themselves to become active. Fibroblast secrete the activators and the inhibitors of MMPs, which allow these cells to participate in regulating extracellular degradation. The most important mechanism for the physiologic turnover and remodeling of collagenous was intracellular degradation. This process involves recognition of the fibrils to be degraded, possibly through binding to fibroblast integrin receptors, partial digestion of the fibrils into smaller fragments, probably by gelatinase A, formation of a phagolysosome, and intracellular digestion of the collagen fragments within the acidic environment of the phagolysosome by lysosome enzymes, particularly the cathepsins.^{11,13}

The essence of mengkudu also helps the availability of xeronine in body. This enzyme is really needed in every metabolism activity in human body, that's why this fruit can strengthen body immunity system and also useful as adaptogen, to balance the work of cells in human body. The enzyme exist in mengkudu fruit in the form of proxeronine

compound.² Sorbic acid in mengkudu fruit is the abundant source of vitamin C, sorbic acid is essentially needed by fibroblast to produce collagen.¹⁴ Vitamin C has a crucial role in wound recovery process as first antioxidants defence in plasma against reactive oxygen species (ROS) and free radicals, which can heavily damage cell and interfere with wound recovery process. The escalation of ROS will cause damage in DNA which will cause cell death.¹¹ Another contents such as terpenoids and flavanoids have been known in their role as astringent to help wound recovery process, which is actively seen in wound contraction and increasing of epithelization process.^{15,16}

In conclusion, the application of mengkudu gel can accelerate the escalation of fibroblast amount after the tooth extraction on Dawley rats. It is showed that mengkudu gel was a better modulator to recovery from damaged tissue.

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