

Effectiveness of Kirinyuh (*Chromolaena Odorata*) Extract on Increasing of Collagen Fibers after Tooth Extraction

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Abstract

Tooth extraction is procedure of removing a tooth from the socket. Kirinyuh leaves (*Chromolaena odorata*) contain flavonoids, saponins, and tannins that can help in the wound-healing process after tooth extraction. This study aimed to determine the effect of applying kirinyuh leaf ethanol extract on the density of collagen fibers in the wound after tooth extraction in guinea pigs.

Sixty male guinea pigs (*Cavia cobaya*) were divided into five groups: negative control, positive control, and treatment groups with kirinyuh leaf ethanol extract concentrations of 2.5%, 5%, and 10%. Extracts of kirinyuh leaf were made as topical products. Subjects' teeth were extracted on the same day, and extract of kirinyuh leaves was applied topically on the wound. Subjects were euthanized, and histopathological specimens were obtained on days 3, 7, 10, and 14 after tooth extraction, subjected to Mallory trichrome staining, and then observed under a microscope. The treatment groups had higher density of collagen fibers than the controls. Statistical analysis showed significant differences in all groups and days.

In conclusion, the ethanol extract of kirinyuh leaves can increase the density of collagen fibers in post-tooth extraction wounds, and 10% kirinyuh leaf extract has the highest rate of increasing collagen fiber density.

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Introduction

Tooth extraction is conducted in certain cases, for example, the tooth cannot be maintained anymore, irritates surrounding teeth, or affects other organs. Healthy teeth may be extracted for orthodontic treatment. Tooth extraction leaves a wound and may cause complications such as bleeding, pain, infection, and dry socket¹.

Wound healing is the process by which damaged tissues recover its normal condition². The wound-healing process may occur chemically and naturally³. Wound healing involves numerous cell populations, extracellular

matrix, and soluble mediators such as growth factors and cytokines⁴. Collagen is an essential protein that connects cells and has a central role in the production of extracellular matrix that strengthens tissues and closes the wound^{5,6}.

For centuries, Indonesians have used medicinal plants to overcome health problems⁷, such as *kirinyuh* (siam weed or *Chromolaena odorata*), which is a tropical plant from the *Asteraceae* family. Although this plant is often considered harmful as a weed, some regions in Indonesia, especially the Acehnese people, utilize *kirinyuh* as a medicine, especially to treat wound⁸. Its saponin content may boost collagen production, while its tannin and flavonoid components act as antiseptic and antibacterial agents^{9,10}. Thus, this study aimed to compare the density of collagen fibers after tooth extraction in guinea pigs (*Cavea cobaya*) using 2.5%, 5%, and 10% *kirinyuh* leaf ethanol extracts.

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Materials and methods

Production of kirinyuh leaf extract

The first step was the production of *kirinyuh* leaf powder. This process started with washing destemmed *kirinyuh* leaves with water and then finely slicing them before drying under shade. Then, the dried *kirinyuh* leaves were ground using a blender until they turned into powder. Thereafter, 70% ethanol extract was produced through maceration. Liquid preparation was produced by extracting the vegetal ingredients that had been soaked in 70% ethanol. After 3 days, it was filtered using a filter paper to obtain the filtrate from which the solvent was evaporated using rotary vacuum evaporator in 500°C temperature. Finally, *kirinyuh* leaf ethanol extract was obtained.

Treatment on the experimental animals

Sixty male guinea pigs (*Cavia cobaya*), aged 9–10 weeks and weighed 300–350 grams, were used as experimental subjects. We got guinea pigs from our university's Integrated Research Laboratory. Guinea pigs were first acclimated for 7 days in cages. Individual cages measuring 30 x 40 x 15 cm were given to each guinea pig with a dry bush base and cleaned every day. Guinea pigs were fed with standard foods such as pellets, leaves and water *ad libitum*. Ethical clearance was obtained from the Medical Research Ethics Commission of the Faculty of Dentistry, Universitas Gadjah Mada.

Tooth extraction was done on the mandibular first incisor. After the extraction, *kirinyuh* leaf ethanol extract was applied on the sockets of the treatment group once a day. The subjects were divided into five groups: negative control group, positive control group, and treatment groups with *kirinyuh* leaf ethanol extract concentrations of 2.5%, 5%, and 10%. The guinea pigs were euthanized on day 3, 7, 10, and 14 after treatment, with three guinea pigs in each treatment group and control group. The mandibles were extracted and sterilized using 0.9% NaCl and histologically by Mallory staining.

To calculate the collagen fiber density, three investigators observed the preparations at 400× magnification under a microscope. Collagen fiber density data were averages from six different fields of view. The observation score of collagen fiber density was determined by using an ordinal scale ranging from 0 to 5¹¹, with the following evaluation criteria (Figure 1): 0, no collagen

fibers; 1, collagen fiber density <50%, absence of fibers to the less dense tissue structure, there are many cells, vascularity, and mononuclear cells; 3, collagen fiber density >50%, with a denser tissue structure, absence or slight inflammatory reaction; and 5, normal collagen fibrous density (avascular and acellular).

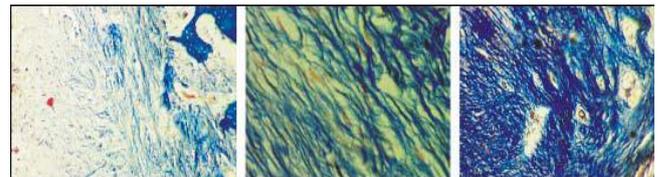


Figure 1. Collagen density score from lowest to highest density (from left to right: scores 1, 2, and 3)^[24].

Statistical analysis

The observation result data were ordinal scale data and analyzed using Kruskal–Wallis tests to know the influence of the number of days and concentration on collagen production, and Mann–Whitney tests were used to see the difference between groups. All data were analyzed using SPSS software (IBM Corp., Armonk, NY).

Results

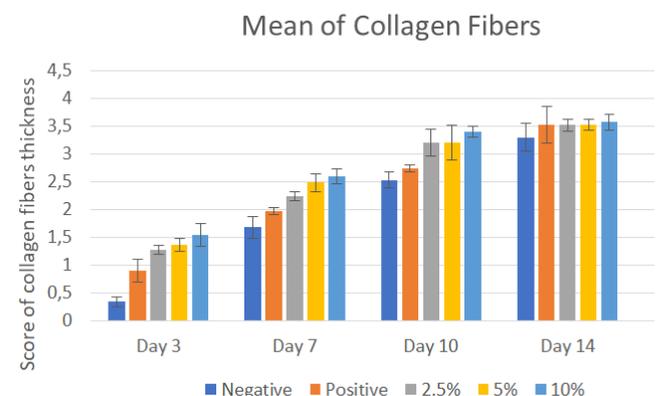


Figure 2. Mean of collagen fiber density.

Figure 2 shows that the means of collagen fiber density on the tooth socket alveolar bones in the negative control, positive control, and 2.5%, 5%, and 10% treatment groups were increasing from the day 3 until 14. Marked increase in the means of collagen fiber density can be seen particularly in the 10% treatment group. Among the groups, 10% *kirinyuh* leaf ethanol extract on the tooth socket on days 3, 7, 10, and 14 had the highest mean collagen fiber density.

On Kruskal–Wallis tests, each group showed significant differences in the density of the tooth socket collagen fibers on day 3, 7, 10, and 14 ($p = .000$). On the Mann–Whitney tests, significant differences were found in the density of collagen fibers between groups during observation ($p < .05$). The topical application of 10% kirinyuh leaf ethanol extract in post-extraction wounds showed earlier formation of collagen fibers than other extract concentrations and control.

Discussion

This study showed that all groups underwent significant increase in collagen fiber density on day 3, 7, 10, and 14 after tooth extraction. Collagen formation is one of the indicators of the wound-healing process. Collagen plays an active role in the proliferation phase by increasing the strength of the wound tissue¹². Cells that play a role in collagen formation include fibroblasts and osteoblasts. Fibroblasts are mesenchymal cells originating from the walls of the alveolar bone and migrate and proliferate in the tooth socket. The increase in collagen fiber density started on day 3 after wounding¹¹, which coincides with the start of the migration and proliferation of fibroblasts. Fibroblasts begins to synthesize collagen on day 3 in response to the transforming growth factor (TGF- β) mediator. Collagen synthesis will reach its peak on days 5–7¹³. Osteoblasts are cells also derived from mesenchymal cells. Osteoblasts synthesize collagen and glycosaminoglycans from the bone matrix and play a role in the bone mineralization process. Osteoblasts secrete several chemical compounds including collagen type 1, alkaline phosphatase, osteopontin, and osteocalcin¹². The increase in collagen synthesis continues until the second week after wounding¹⁴.

Kirinyuh leaf extract toxicity tests have been carried out. The observed toxicity parameter was lethal dose 50 (LD50). That study aimed to determine the LD50 value, or a dose that can kill 50% of mice (*Mus musculus*), and provide a safe dosage data of kirinyuh leaf extract³. Common toxic symptoms are diarrhea and urination. In that study, a dose of ethanol extract from *kirinyuh* leaves of 14.1416 g/kg BW or 28.82% is considered “light toxic”; thus, in the present study, we used extracts at 2.5%, 5%, and 10%, which are considered safe.

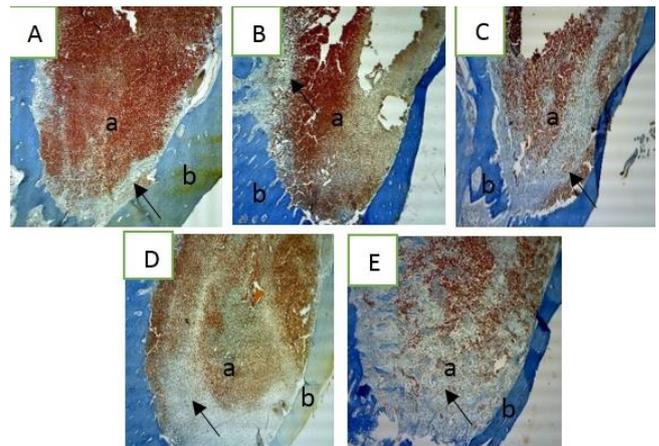


Figure 3. Histological comparison of guinea pig's tooth socket on the apical portion of the socket on day 3 (400 \times magnification). Collagen appears to have formed in all groups, and the thickest collagen density is found in the 10% treatment group. Negative control group (A), positive control group (B), 2.5% treatment group (C), 5% treatment group (D), and 10% treatment group (E). Bone socket area (a), alveolar bone (b), and collagen (arrow).

Figure 3 shows the density of collagen fibers on day 3. Mallory staining shows inflammatory cells and erythrocytes that are scattered over the wound area. Collagen fibers still look very thin and rare. This happens because on day 3 there is overlapping between the inflammatory phase and the proliferative phase of the wound-healing process.

Moreover, on day 3, a significant difference was found between the 2.5%, 5%, and 10% treatment groups compared with the positive control group. These results indicate that the administration of *kirinyuh* leaf ethanol extract gel affected the density of collagen fibers after tooth extraction compared with the positive control group. This finding agrees with the statement of Yenti et al. (2011), who conducted a study of kirinyuh leaf ethanol extract cream with the same concentration in wounds on the backs of mice¹⁵. They found that 2.5%, 5%, and 10% ethanol extract cream showed faster wound-healing rate than 10% povidone-iodine.

The insignificant results between the 2.5%, 5%, and 10% kirinyuh leaf extract concentrations on day 3 indicates that the kirinyuh leaf concentrations have almost the same effect on the formation of collagen. The negative control group showed a significant difference in collagen fiber density from the positive control group and

all treatment groups. The wound-healing process in the negative control group is no better than 10% povidone-iodine or kirinyuh leaf extract because aquades have no other active component that can help speed up the wound-healing process.

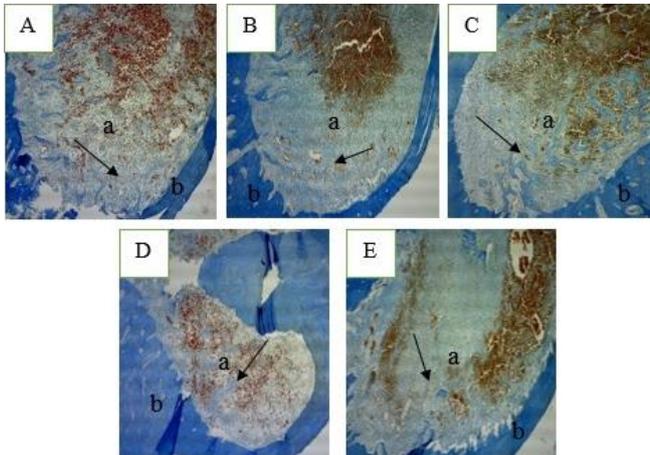


Figure 4. Histological comparison of guinea pig's tooth socket on the apical portion of the socket on day 7 (400× magnification). Collagen appears to have thickened in all groups. Negative control group (A), positive control group (B), 2.5% treatment group (C), 5% treatment group (D), and 10% treatment group (E). Bone socket area (a), alveolar bone (b), and collagen (arrow).

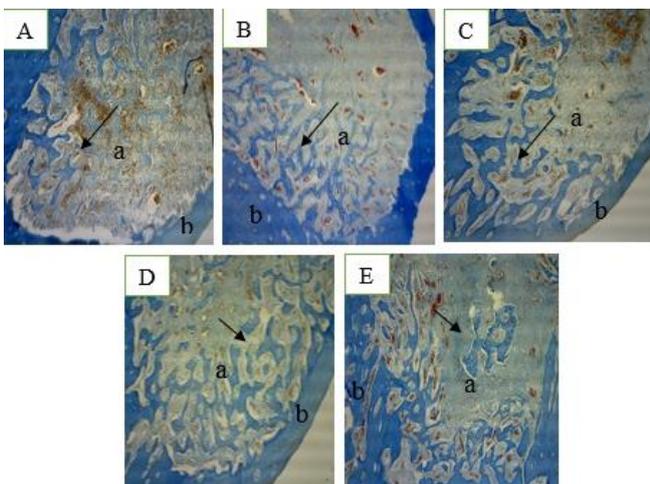


Figure 5. Histological comparison of guinea pig's tooth socket on the apical portion of the socket on day 10 (400× magnification). Collagen has partially mineralized into the bone. Negative control group (A), positive control group (B), 2.5% treatment group (C), 5% treatment group (D), and 10% treatment group (E). Bone socket area (a), alveolar bone (b), and collagen (arrow).

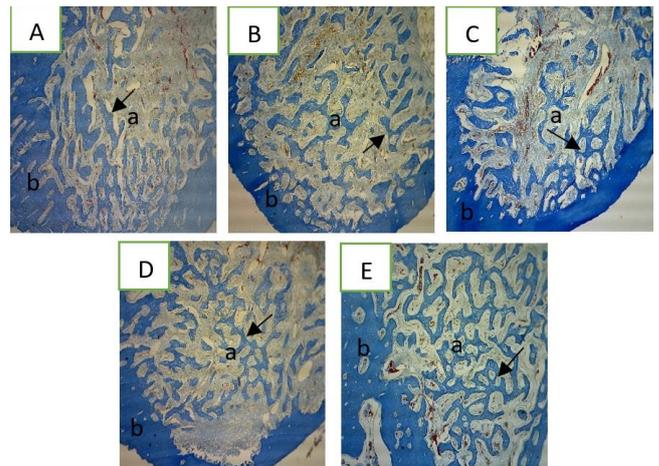


Figure 6. Histological comparison of guinea pig's tooth socket on the apical portion of the socket on day 14 (400× magnification). Mineralization appears to have formed in all groups, and the 10% treatment group showed a wider area than the other groups. Negative control group (A), positive control group (B), 2.5% treatment group (C), 5% treatment group (D), and 10% treatment group (E). Bone socket area (a), alveolar bone (b), and mineralized collagen (arrow).

Observations on days 7–10 show that all groups had higher collagen fibers than on day 3 (Figures 3–6). Fibroblasts start appearing significantly for the first time on day 3 and will reach their peak on day 7¹⁶. Fibroblasts are deposited in the extracellular matrix in the form of collagen fibers that will peak on day 7. Fibroblasts produce large amounts of collagen. This collagen is in the form of triple glycoprotein, the main element of extracellular wound matrix which is very useful for the strength formation of scar tissue¹⁷. Saponin compounds in *kirinyuh* leaf ethanol extract may help increase collagen fiber density by stimulating fibronectin synthesis. Increasing fibronectin synthesis will accelerate the migration and proliferation of fibroblasts to the wound area; thus, the collagen synthesis will increase¹⁰.

In this study, no significant difference was found on day 7 between the 25% treatment group and positive control group. This occurs because both groups have components that can accelerate wound healing. Povidone-iodine is a local antibacterial compound that is effective in killing bacteria and spores and is widely used for antiseptics. Povidone-iodine, which is used as a positive control, can kill infection-causing microorganisms, such as Gram positive bacteria

and Gram negative bacteria including spores and fungi. Two mechanisms can explain the antimicrobial effect of 10% povidone-iodine: povidone-iodine can oxidize enzymes for respiration, and it has antimicrobial effect through iodination of amino acids. The iodine content prevents bacteria from forming proteins and destroys microorganisms¹⁸. *Kirinyuh* leaf ethanol extract also contain an antibacterial component, that is, saponins. Saponins can increase the permeability of bacterial cell membranes. Permeability is the ability of a substance to allow passage of particles through it¹⁹. When saponins and bacteria interact, saponins increase the permeability of bacterial cell membranes to allow passage of porous forms in bacterial cells. Eventually, the cell will undergo lysis and die following loss of all cell contents that diffuse out of the cell²⁰.

Microscopic observations on day 10 showed the presence of increasingly dense collagen in all groups. The density of collagen fibers is increasing and reaches its peak on day 10 after application of *kirinyuh* leaf ethanol extract. Changes in the density of collagen fibers in the treatment group show an earlier bone matrix formation process marked by the formation of collagen bundles. The maturation of collagen fibers causes an increase in the density of collagen fibers in tissues^{13,21}. The increase in the thickness of collagen was attributed to the gel content of *kirinyuh* leaf ethanol extract which can stimulate the formation of collagen, affecting the proliferation phase after tooth extraction. The content of *kirinyuh* leaf ethanol extract that can induce cells that play a role in the healing process of wound healing include flavonoids, saponins, and tannins.

The increase in collagen thickness is allegedly caused by the contents of *kirinyuh* leaf ethanol extract that boost collagen production and affect the proliferation phase in the wound-healing process after tooth extraction²². According to Youngyo et al. (2017), flavonoids have antioxidant properties, so they can reduce low-density lipoproteins²³. Flavonoids also improve the number of blood vessel endothelial cells by reducing the risk of blood clots because of their anti-aggregation properties. Tannin can be defined as a polyphenol compound with a very large molecular weight (>1000 g/mol) and can form complex compounds with protein. Tannin has a great biological role because of its

function as a protein precipitator and chelating metal. Tannin is predicted to act as a biological antioxidant²⁴. Saponin may increase the fibroblast's receptor ability to TGF- β , given the ability of fibroblasts to bind with TGF- β . Fibroblasts need TGF- β to synthesize collagen to heal the tooth socket wound¹⁰. Saponin has anti-inflammatory activity by blocking the prostaglandin production pathway, resulting in a decrease in prostaglandin production²⁵. Decreased production of prostaglandins, an inflammatory mediator, can ease the vasodilation in blood vessels in the local bloodstream so that the migration of inflammatory cells will decrease. Decreased number of inflammatory cells and bacteria causes brief inflammatory phase and immediately initiates the proliferation phase^{26,27}. This may cause the matrix classification to occur earlier.

Observation on day 14 shows that the density of collagen fibers treated with 10% *kirinyuh* leaf ethanol extract has the highest mean among the groups. Figure 6 shows that bone mineralization occurred mostly in the tooth socket wound in the 10% treatment group. On day 14 after tooth extraction, collagen fibers have undergone mineralization and reinforcement has started. On day 14, collagen fibers are ready to enter the maturation phase. The maturation phase starts 2–3 weeks after wounding. In this phase, the collagen level is stable between deposition and degradation^{14,28}. Enzymes such as matrix metalloproteinase, cysteine proteinase, and serine proteinase can degrade collagen fibers in the alveolar bone. This indicates that the bone-modeling process is ongoing, which marks that first stage of bone regeneration.

Conclusions

Topical application of *kirinyuh* leaf ethanol extract can accelerate wound healing, as shown by the increase in collagen fibers on the wound after tooth extraction. The highest increase in collagen density was observed after the application of 10% *kirinyuh* leaf ethanol extract. Thus, 10% concentration of ethanol extract from *kirinyuh* leaf helps accelerate the wound-healing process since days 3–4 of tooth extraction.

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Declaration of Interest

The authors report no conflict of interest.

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