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The effectiveness methanol extract clausena excavate on number of fibroblast and density of collagen fibers after tooth extraction

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Abstract

Objective: This study is made to identify the effect of extracts in Clausena excavata 50% on the number of fibroblasts and the density of collagen fiber in recovery after tooth extraction.

Material and Methods: This is a true experimental designed with post-test only control group design, using 24 rats were divided into two groups, each group of three rats. Lower incisor tooth extraction performed lower left, into the tooth socket CMC applied to the control group and 0.5% extract treatment group Clausena excavata 50%. The number density of fibroblasts and collagen fibers were observed on days 3, 7, 14 and 21 with Haematoxylin Eosin staining and Mallory. The differences in density of collagen fibers observation group with Kruskal-Wallis test.

Results: There are significant differences in a number of fibroblasts and density of collagen fibers between a control group and socket treatment group ($p < 0.05$).

Conclusion: The extract Clausena excavata 50% can fasten the wound recovery by increasing the number of fibroblasts and collagen fibers.

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Introduction

Tooth extraction damages tissue around the socket, In this conditions the body seeks to improve the integrity and capacity of the tissue functions, both cell and biochemical called wound healing. The process of wound recovery is complex and dynamic, continuous, overlapping, with the correct time, consisted of phases of inflammation, proliferation and remodeling.[1]

Inflammatory phase takes place since the injury until the fifth day.[2] This phase is dominated by neutrophil cells to fight infections and cleans cellular matrix debris and unidentified objects. The existence of persistent neutrophils cause difficulty on wounds to heal. It can cause acute injury which can be chronic wounds,[3] if the wound is not infected, neutrophil numbers decline, subsequently phagocytosis followed by macrophage. The presence of macrophages will increase the osteoclasts activity. Osteoclasts scrape the surface of the socket's wall. Macrophages secreting proinflammatory cytokines, anti inflammatory and growth factors such as tumor necrosis factor α (TNF-α), transforming growth factor β (TGF-β), interleukin 1 (IL-1), interleukin 6 (IL-6), interleukin 8 (IL-8)), proteinase (Collagenase Enzyme), Metalloproteinase Matrix (MMPs) and prostaglandin E2 (PGE2), which degrade extracellular matrix remaining, stimulates the movement, differentiation and proliferation of cells for recovery of damaged tissue.[3]

The phase of proliferation is started from day 3 to day 21, characterized by change of provisional matrix which is dominated by platelets and granulation tissue (new blood vessel / angiogenesis, fibroblasts, and macrophages). Angiogenesis is important because it supplies the wound area with oxygen, nutrients and important mediator for wound healing. During angiogenesis in endothelial cells secrete growth factors of Vascular Endothelial Growth Factor (VEGF), fibroblast growth factor (FGF), TGF-β and Angiopentin. These growth factors stimulate endothelial cells for angiogenesis. After the formation of the tissue is adequate, migration and proliferation of endothelial cells decreases, excess cells will become apoptosis.[4] Concurrently with the angiogenesis, keratinocyte cells proliferate and migrate to form epithelium to cover the injured area.[5]

In the proliferation phase, macrophages produce growth factors PDGF, FGF and TGF-β which induce fibroblasts to proliferate, migrate, and produce extracellular matrix (Collagen, Elastin fibers and Reticular fibers). These fibers fill wound cavity and

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provide scallops for keratinocyte cell migration and osteoblasts.[4] Further fibroblas degrade the fibrin matrix and replace it with a glycosaminoglycan (GAG). Fibroblast begins to proliferate 24 hours after injury, the number increased on day 3, a peak at day 7, and the numbers continue to decline.[6]

Collagen is the main protein that make up components of extracellular matrix, is required in wound healing, scarring, and creating of bone matrix.[7] In wound healing, Collagen is formed since the 3rd day, peak day 7th and day 14th the number will decline.[1]

Pharmaceutical technology development has led to fitofarmaka, thus standardized herbal medicine. One of the herbs that used by society is the Koja bay leaves (Clausena excavata). Phytochemical test shows that this plant contains flavonoids, saponins, steroids, triterpenoids, phenolics and tannins, which have an effect as an antioxidant, anti-inflammatory, antibacterial, antifungal, and non-aseptic.[8]

Based on the above, researchers want to identify the effect of giving a leaf extract Clausena excavata to the number of fibroblasts and density of collagen fibers after tooth extraction. Pre-experimental that has been made that the application of the methanol extract of leaves Clausena excavata into the socket after tooth extraction with a concentration of 20%, 50% and 100%. Based on the pre-experimental results of research the most effective concentration is 50%, therefore, in this study it can be used on extracts Clausena excavata concentration of 50%.

Material and Methods

This is a purely experimental research with post test only control group design, it has been underway in December 2017 to March 2018. Identification and manufacture of extracts performed in the laboratory of Chemistry, University of Padjadjaran, tooth extractions of animals research and extract in the Integrated Research and Testing Laboratory of the University of Gajah Mada and manufacture of paraffin blocks and observations in the Laboratory of Pathology Faculty of the University of Gajah Mada. Research declared eligible to conduct by the Research Ethics Commission of the Faculty of Medicine, University of Andalas No: 541 / KEP / FK / 2017.

Twenty four Galur Wistar white rats, aged 6-7 weeks with a weight of 200-250 mg, divided into 2 groups. In the control group, the application of CMC 0.5%, In the treatment group, the application of methanol extract Clausena Excavata concentration of 50% that diluted with 0.5% CMC. Rats adapted for a week, then carried down to the left incisor tooth extraction, after the tooth extraction into the socket, each group is given 0,1cc once a day for three days. Animals terminated on days 3, 7, 14 and 21, which there be preparation made for observation HE histological staining fibroblast and Mallory's collagen fibers.

Scoring parameters histopathological fibroblasts: 0 = Not found the fibroblasts; + 1 = Number of fibroblasts in the wound area is low (<10% per field of view); +2 = The number of fibroblasts in the wound area medium (10 s / d 50% per field of view); + 3 = Number of fibroblasts in the wound area "narrow"(50 s / d 90% field of view); +4 = Number of fibroblasts in a very narrow wound area (90 s / d 100% field of view). Scoring collagen fibers: 0 = Not found collagen fibers; +1 = density of collagen fibers in the injured area (<10% per field of view); +2 = density of collagen fibers in the wound area medium 10% s / d 50% per field of view); +3 = density of collagen fibers in the injured area narrow (50% s / d 90% per field of view); +4 = density of collagen fibers in the injured area is very tight 90% s / d 100% per field of view. the observations were made in the apical 1/3, 2/3 and 1/3 cervical apical, with 400x magnification).

The data were tested with Kurskal-Wallis to determine the differences in the number of fibroblasts and collagen fibers of the second group of observations,

Results

The observation of the number of fibroblasts from both groups of observations showed the following results:

The observation of the density of collagen fibers of the second group of observations showed the following results:

Data obtained from both groups observation Kruskal-Wallis test and obtained the value of significance ($p < 0.05$).

Kruskal-Wallis test results showed a significant difference in both groups observation days 3, 7, 14 and 21 ($p < 0.05$).

Discussion

Clausena excavate is one kind of plant that contains flavonoids, saponins, and tannins. Wound healing can be accelerated by administration Clausena excavate extract.

In this study the average number of fibroblast cell formation and collagen fibers was higher in the treatment group than in the control group. Formation of peak fibroblasts on the 7th day. This finding is consistent with the theory that fibroblasts begin to form on day 3, fibroblasts have increased

Table 1 Kruskal-Wallis test fibroblasts control and treatment groups on day 3, 7, 14, and 21

Day	Control / treatment	N	Mean Rank	Sig.
3	CMC	3	5.00	
	Clausena excavate 50%		7.00	
7	CMC	3	17.50	
	Clausena excavate 50%		21,50	0.011
14	CMC	3	14,00	
	Clausena excavate 50%		17.50	
21	CMC	3	7.00	
	Clausena excavate 50%		10.50	

Table 2 Kruskal-Wallis test collagen control and treatment groups on day 3, 7, 14 and 21

Day	Control / treatment	N	Mean Rank	Sig.
3	CMC	3	5.50	
	Clausena excavate 50%		12.50	
7	CMC	3	16.00	
	Clausena excavate 50%		23.00	0.049
14	CMC	3	9.00	
	Clausena excavate 50%		12.50	
21	CMC	3	9.00	
	Clausena excavate 50%		12.50	

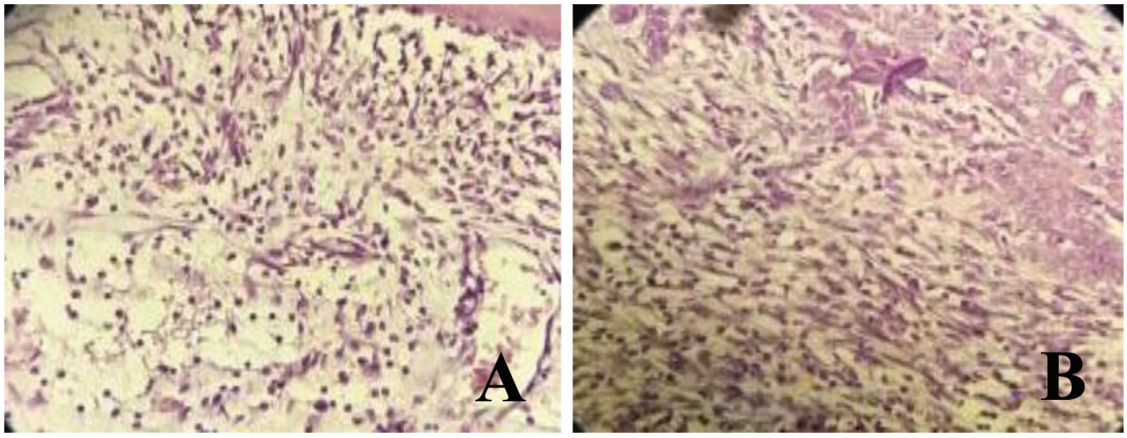
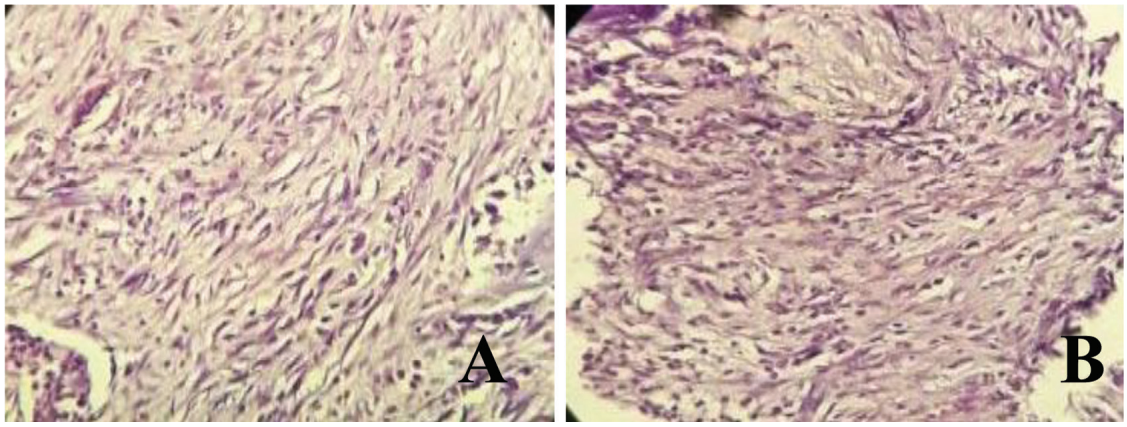


Figure 1 Histopathologic features fibroblast cells on the 3rd day. The control group, A. Treatment group, B. (HE staining, magnification 400x)



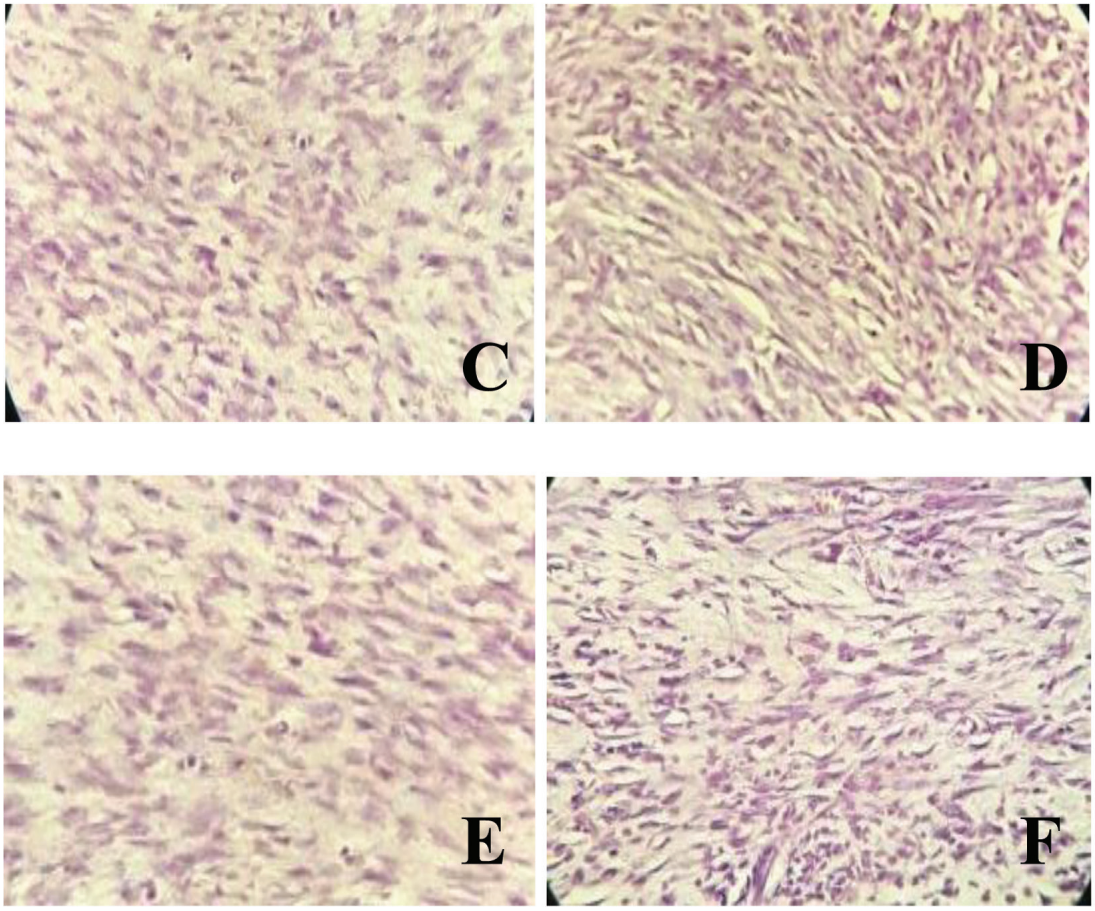
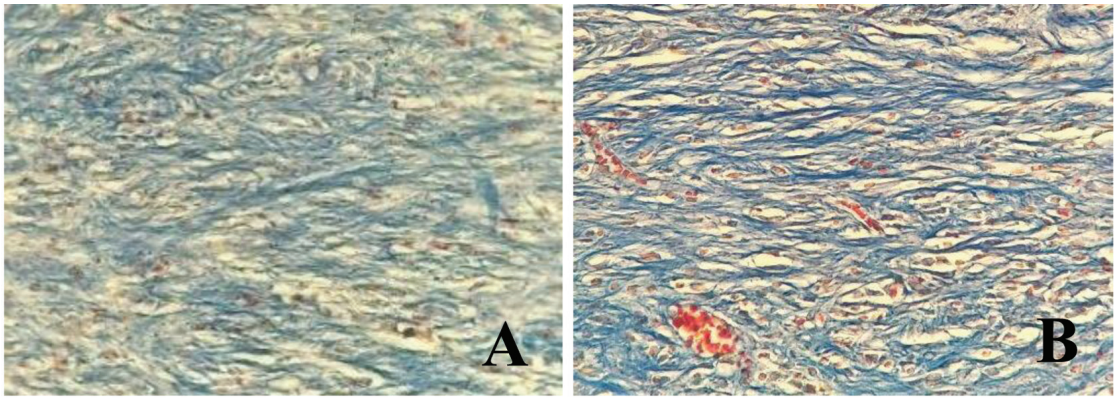


Figure 2 Histopathologic features of fibroblasts on the 7th day of the control group, A. Treatment group, B. (HE staining, magnification 400x); Histopathologic features of fibroblasts on the 14th day of the control group, C. Treatment group, D. (HE staining, magnification 400x); Histopathologic features of fibroblasts on day 21 the control group, E. Treatment group, F. (HE staining, magnification 400x)



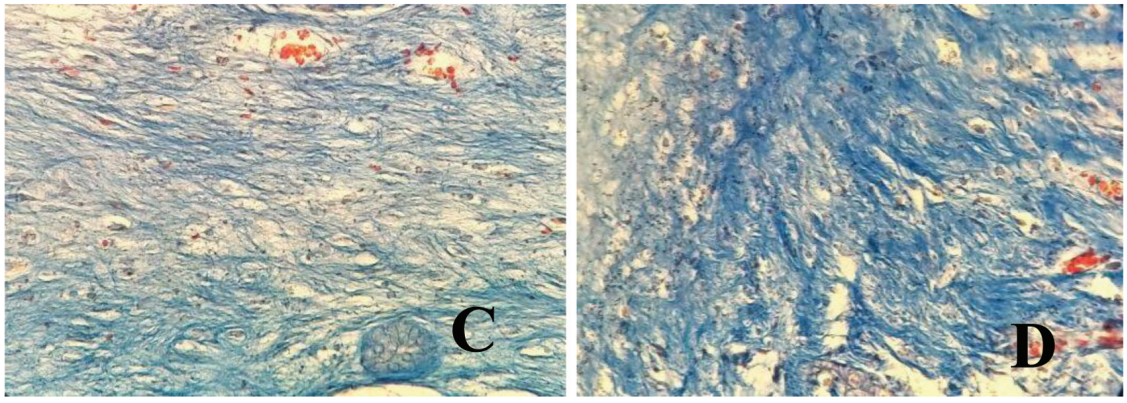


Figure 3 Histopathologic features of collagen fibers day 3 in the control group, A. Treatment group, B. (Mallory staining, magnification 400x); Histopathologic features of collagen fibers day 7 in the control group, C. Treatment group, D. (Mallory staining, magnification 400x)

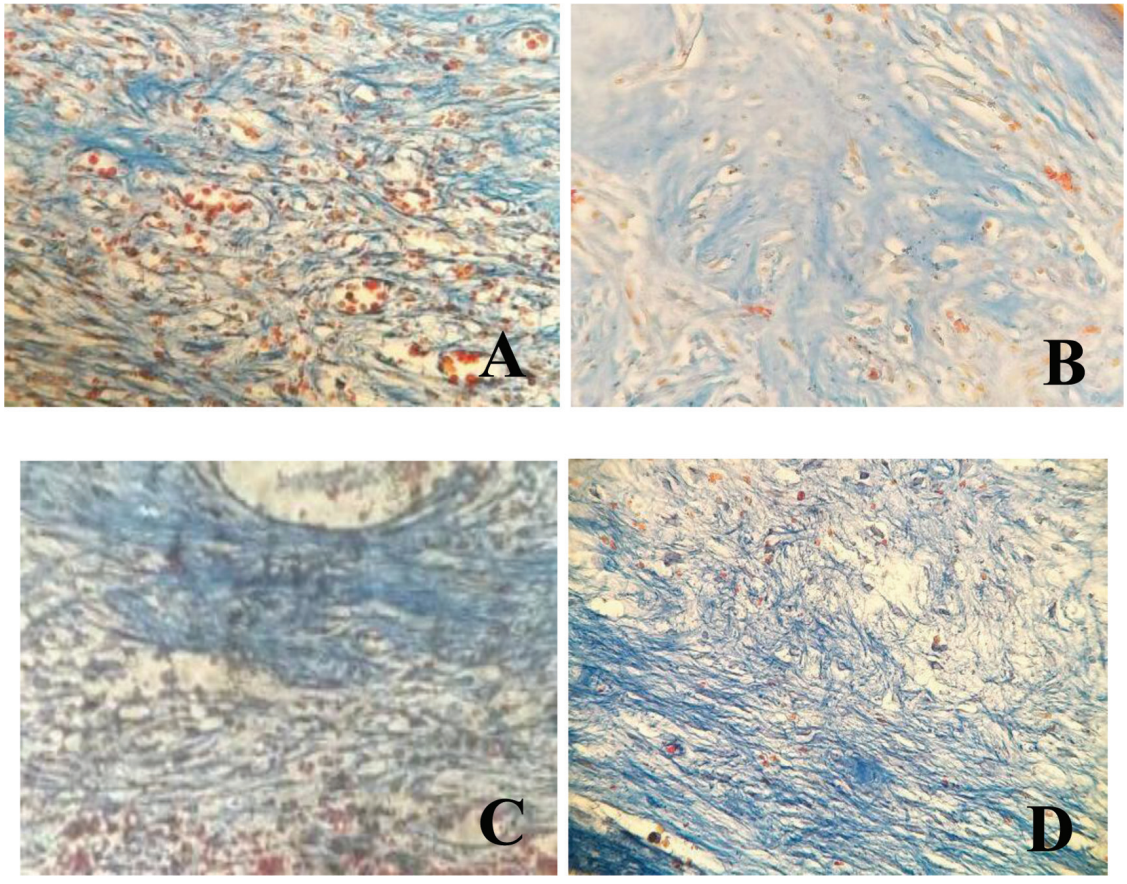


Figure 4 Histopathologic features of collagen fibers day 14 in the control group, A. Treatment group, B. (Mallory staining, magnification 400x); Histopathologic features of collagen fibers day 21 in the control group, C. Treatment group, D. (Mallory staining, magnification 400x)

to a peak on day 7, namely the proliferation phase. On the 14th day the number of fibroblasts begins to decrease and on the 21st day the number decreases, this can be caused by the synthesis of collagen by fibroblasts to link the wound has mediated and some fibroblasts turn into fibrocytes.[9]

The formation of collagen fibers begins on the 3rd day and peak day 7, declining at day 14 to 21. This finding is consistent with the theory that the creation of collagen fibers appear on day 3 because in this phase of the migration and proliferation of fibroblasts will form collagen fibers. The formation

of collagen at day 14 and day 21 decreased, this may cause collagen is replaced bone matrix. In the proliferative phase, collagen created is type III in line with the passage of time in the phase of type III collagen remodeling being replaced by collagen type I in the form of a ribbon and has a tensile strength that is more powerful, which will give you the strength and density of the new network.[10]

Increasing the number of fibroblasts in the wound tooth extraction by Clausena excavate extract can be caused by compounds contained in Clausena excavate extracts are flavonoids, saponins and tannins. Flavonoids can help wound healing by increasing the formation of collagen and fibroblasts and decrease tissue edema. Saponins can increase the number of macrophages migrate to the injured area will secrete cytokines and growth factors that will degrade extracellular matrix is left, stimulates migration, differentiation and proliferation of endothelial cells, fibroblasts, keratinocytes and osteoblasts, to the injured. Osteoblast cells will aggregate the intercellular substance of bone containing collagen to form new collagen fibers and form osteoid.

Extract Clausena Excavata contains flavonoids, saponins, and tannins. Flavonoids are antioxidants that can reduce the peroxidase, increasing the speed of epithelialization, and antimicrobials. The decrease lipid peroxidation by flavonoid prevents necrosis, improve vascularization, and improve the viability of collagen.[11]

Saponins are chemical substances that are useful in influencing collagen (the early stages of tissue repair) which inhibits the production of excessive scar tissue.[12] Saponin have a high degree of toxicity against fungi, thus helping the healing process.

Tannins play a role in cleaning up free radicals, enhance wound closure, and improves capillary blood vessel formation as well as fibroblasts.

Conclusion

Clausena excavate leaf extract 50% effect on increasing the number of fibroblasts in wound healing post extraction sockets. With increasing fibroblast cells, it also increases the synthesis of collagen and extracellular matrix which is used to replace tissue damaged by tooth extraction.

Acknowledgment

None.

Conflict of Interest

The authors report no conflict of interest.

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