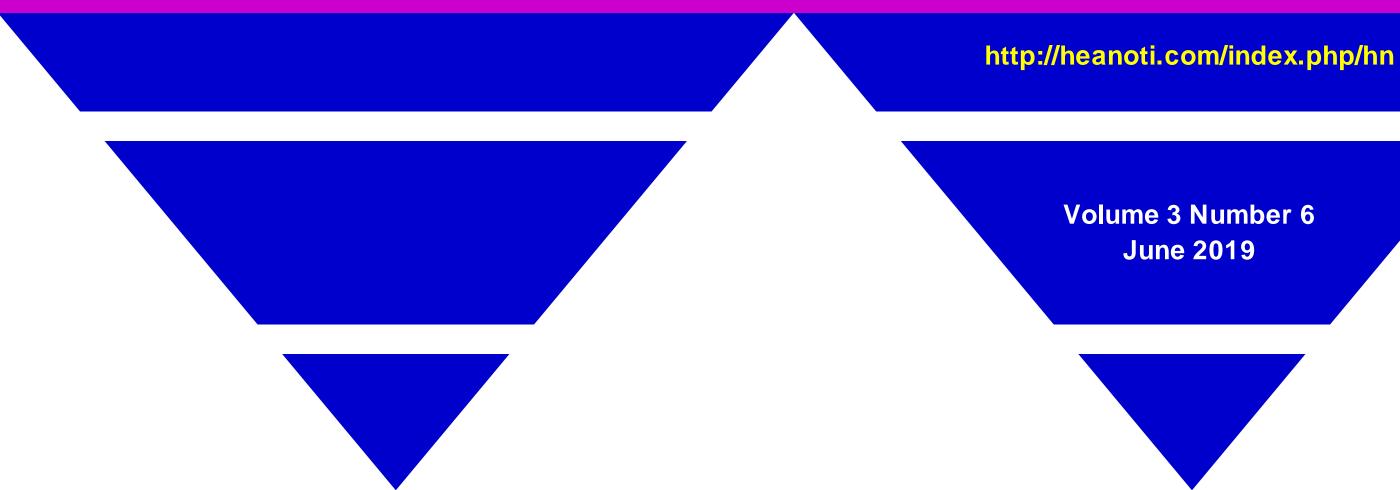


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# **Health Notions**

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9. References written with a hanging indentation 1 cm, referring to the Vancouver style.

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# Arabica Coffee (*Coffea Arabica*) Fruit Skin Potential Towards the Increase of Fibroblast Cells Amount Within Socket Post Tooth Extraction of Male Wistar Mouse

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**Background:** Tooth extraction can cause wound in hard tissue and soft tissue inside oral cavity. Wound healing process involves cell proliferation especially fibroblast cells. To accelerate fibroblast cell proliferation alternative substance that can accelerates wound healing process is needed which is arabica coffee fruit skin. Arabica coffee fruit skin contains substances such as polyphenols, tannin, chlorogenic acid, and caffeine expected to be able to stimulate fibroblast cells amount. **Objective:** To understand arabica coffee fruit skin potential towards increase in number of fibroblast cells on the socket after tooth extraction. **Method:** The design of this research was post test only control group with sample size of 24 wistar mouse. The samples used were divided into two groups which are control and treatment group. Then treatment was given in 3 days, 5 days, and 7 days. Then they were decapitated to undergo tissue processing with HE coloring and post tooth extraction socket fibroblast cells counting. Acquired data then analyzed used One Way ANOVA and LSD test. **Result:** Fibroblast cell amount increased significantly (p<0.05) in treatment group compared to control group. **Conclusion:** Arabica coffee fruit skin potentially increase fibroblast cells amount in socket post tooth extraction of male wistar mouse.

Keywords: tooth extraction; wound healing; fibroblast cells

#### INTRODUCTION

#### Background

Tooth extraction is a process to remove tooth from alveolar, where the said tooth can no longer receive treatment. Tooth extraction can cause a wound to appear at either soft or hard tissue<sup>(1)</sup>. Wound healing process post extraction involves inflammatory phase, proliferation phase, and remodeling phase. Inflammatory phase is a first reaction when body receive lesion. Several minutes after, vasodilation and capillary permeability raise occured because of release of chemicals at wounded tissue. And then next is proliferation phase which is a phase where new blood vessel and granulation tissue are formed. Fibroblast cells are cells with important role in this phase. In wound healing process, fibroblast cells first appeared on the third day and reach peak on day 7. Fibroblast cells responsible to pruduce protein structure that will be used during tissue reconstruction process like collagen, elastin, and reticular. Remodeling/maturation phase is the longest phase<sup>(2,3,4)</sup>.

Technology development of health pharmacy in the world has centered their attention to use material that are produced from nature because it is safer to use compared to medicine that contains chemicals. One of natural material currently developed is arabica coffee (*Coffea arabica*). Arabican coffee is a type of coffee that is the most consumed which is 70%<sup>(5)</sup>. Coffee production also produced waste as in coffee skins that is not used optimally in health department. Content of coffee fruit skin isn't much of a different compared to coffee fruit itself, which contain polyphenols, tannin, chlorogenic acid, and caffein. Coffee fruit has been researched with one of the result is for incision wound recovery<sup>(6,7)</sup>.

Arabica coffee fruit skin contain higher polyphenols than robusta coffee. Coffee fruit skin contain caffein and polyphenols that can function as natural antioxidant. Polyphenols covers flavonoids, catechins, epicatechins, procyanidin, anthocyanin, complex tannin, and flavonol glycosides. Flavonoids function as anti-inflammatory. Flavonoids inhibit important phase in prostaglandin biosynthesis, in cyclooxygenase pathway so inflammation process is shorter and fibroblast proliferation is faster, so it triggers collagen formation and accelerate wound healing process<sup>(7,8,9,10)</sup>.

#### Objective

The objective of this research is to understand potention of arabica coffee fruit skin (*Coffea arabica*) towards the increase of fibroblast cells amount in post tooth extraction socket of male wistar mouse.

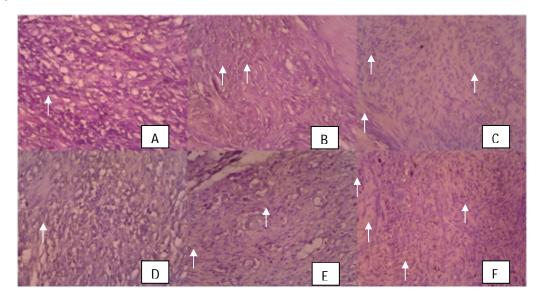
#### METHOD

The research type was laboratory experimental with post test only control group design<sup>(11)</sup>. This research was conducted in the biomedical laboratory of the faculty of dentistry, Universitas Jember in August until November 2018. Sample size were 24 male Wistar mouse with age 2-3 month old and body weight 200-225 gram separated into 2 groups named control and treatment group. Treatment group given arabica coffee fruit skin powder with intragastric method and dose of 0.117 gr/day for 3 days, 5 days, and 7 days. Control group given akuades 2 ml intragastrik for 3 days, 5 days, and 7 days. Male Wistar mouse decapitated at day 4, 6, and day 8 to execute tissue processing with HE coloring, next fibroblast cells in tooth socket post extraction observed using optilab that was connected with binocular microscope with 400X magnifier. Data then tested using One Way ANOVA test with 95% confidence level. And next tested with post hoc Least Significant Difference test to see differences between each groups.

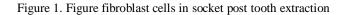
#### RESULT

#### **Fibroblast Cells Amount**

According to Figure 1, tooth extraction socket was seen with granulation tissue inside that was made of fibroblast cells. Fibroblast cells were cells with flat shape with oval cores. Fibroblast cells observed using *optilab* that was connected to binocular microscope with 400X magnifier. Fibroblast cells picture as follows (Figure 1).



Explanation: (A) Histology picture of fibroblast cells in tooth socket post extraction from Control group day 3 with 400X magnifier, (B) Control group day 5, (C) Control group day 7, (D) Treatment group day 3, (E) Treatment group day 5, (F) Treatment group day 7, and (G) White arrow marks shows fibroblast cells inside socket.



Based on observation at preparation socket post male wistar mouse tooth extraction, we obtained average and standard deviation of fibroblast cells amount at research group (Tabel 1).

Group	3 days		5 days		7 days	
Sample	Mean	SD	Mean	SD	Mean	SD
Control	10.22	2.63	17.13	0.97	29.91	4.77
Treatment	18.91	1.62	24.22	2.33	43.58	5.91

Tabel 1. Mean and standard deviation of fibroblast cells amount

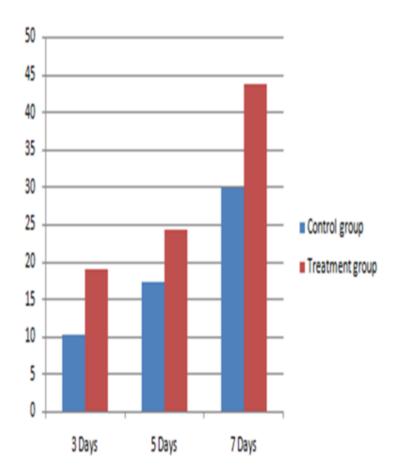


Figure 2. The mean of fibroblast cells amount of control group and treatment group in socket post tooth extraction

According to Tabel 1 and Figure 2, showed a raise of fibroblast cells amount in control group and treatment group. However, fibroblast cells amount in treatment group were higher compared to control group.

#### **Data Analysis**

Normality test result used Shapiro-Wilk test showed significance value (p>0.05) and for homogeneity test used Levene achieved significance value p<0.05. Based on test result, distributed data were normal but inhomogeneous. Next, One Way ANOVA test was done to see if there was a difference between average of fibroblast cells amount between control and treatment group. Based on One-way ANOVA test we achieved significance value of (p<0.05). This showed that there was a significant difference between control and treatment group. Next, post hoc LSD (Least Significant Difference) done to see if there was a differece between each research group with significance (p<0.05). Summary of One Way ANOVA and LSD as follows in Tabel 2.

Group	n	3 days	5 days	7 days	Р
Control	4	10.22±2.63 <sup>aA</sup>	17.13±0.97 <sup>aB</sup>	29.91±4.77 <sup>aC</sup>	0.000*
Treatment	4	18.91±1.62 <sup>bA</sup>	24.22±2.33 <sup>bB</sup>	43.58±5.91 <sup>bC</sup>	0.000*
Р		0.003*	0.010*	0.000*	

Tabel 2. Summary of data analysis result of fibroblast cells amount between each group

Explanation :

(\*): There was a significant difference (p<0,05);

(ab) : Superscript in a column shows significant difference;

(ABC) : Superscript in a row shows significant difference between each group.

#### DISCUSSION

Wound recovery process starts with inflammatory phase, inflammatory phase is the first reaction after wounded. Blood vessels and lymph vessels undergo vasoconstriction several minutes after trauma. Platelets aggregates around wounded endothelium to form blood clot. Then, blood vessels vasodilation occur because of inflammatory mediator release such as prostaglandin in wounded tissue. Next, proliferation phase is an important phase in wound recovery. In this phase, fibroblast cells is the cell with important role. Fibroblast cells is the main component for connective tissue. Fibroblast cells first appear on the  $3^{rd}$  day and reached peak on  $7^{th}$  day<sup>(3,12)</sup>.

Based on research result, the average of fibroblast cells amount in socket post tooth extraction of male wistar mouse in control group during 3 days until 7 days keeps raising. And also the same for treatment group. Fibroblast cells amount in treatment group are higher compared to control group. Average of fibroblast cells amount in treatment group has a significant raise during 3 days compared to control group during 3 days. Treatment group has a significant raise during 5 days compared to control group during 5 days. While, during 7 days treatment group also has a significant raise in average amount of fibroblast cells compared to 7 days on control group.

Arabica coffee fruit skin contains many compound with some of them being caffeine, chlorogenic acid, tannin, and polyphenols. Those compounds synergize with each other to accelerate fibroblast cells proliferation that helps healing process in extraction tooth socket. Contents of arabica coffee fruit skin which are caffeine and chlorogenic acid plays a role in wound healing process. Caffeine and chlorogenic acid are an antioxidant. Having antioxidant helps body to prevent damage by radical compounds, such as decreased immune system. Caffeine also function as antibacterial by inhibiting bacterial activity by enhancing lysozyme activity. Chlorogenic acid belongs to ester family which is formed from the bond of quinine acid, and few transinamic acid, usually caffeic, pcoumaric, and ferulic acid. Chlorogenic acid om arabica coffee fruit skin is able to

stimulate macrophages to release *growth factor* (TGF- $\alpha$ , TGF- $\beta$ , PDGF, VEGF, and IL-1) to help accelerating healing process. TGF- $\beta$  plays a role in angiogenesis, reepithelialization, and tissue bond regeneration<sup>(13,14,15)</sup>.

Flavonoids are the biggest group in polyphenols. Flavonoids in arabica coffee fruit skin powder plays important role to accelerate wound healing process. Flavonoids able to inhibit arachidonic acid by blocking lipoxygenase and cyclooxygenase path. Arachidonic acid then will synthesize prostaglandin and thromboxane through siklooksigenase path, while leukotriene will be produced through lipoxygenase path. If arachidonic acid is inhibited, then the production of prostaglandin, thromboxane, and leukotriene as inflammatory mediator will decrease. That will cause the decrease of sore pain, edema, and blood vessel vasodilation. Then, inflammatory cell migration to wounded area will decrease and inflammation process will shorten and immediately enter proliferation phase<sup>(10,16)</sup>.

Flavonoids and tannin able to raise TGF- $\beta$  so TGF- $\beta$  can induce fibroblast cells migration into fibrin matrix<sup>(17)</sup>. Macrophages will keep producing growth factor such as PDGF and TGF- $\beta$  that induce fibroblast to proliferate, migrate, storing extracellular matrix, and stimulate endothelium cells to form new blood vessel. With so many activated TGF- $\beta$ , the amount of fibroblast cells that migrate to wounded part will increase. The more fibroblast cells formed, collagen fibers will also increase so that healing process will accelerate. Not only collagen fiber, said fibroblast cells will then form connective tissue by forming reticular fiber, elastic, glycosamine, and glycoprotein which is the base components of connective tissue<sup>(18)</sup>.

#### CONCLUSION

Based on research result, it can be conluded that compound content of arabica coffee fruit skin (*Coffea arabica*) which are polyphenols, tannin, chlorogenic acid, and caffeine synergize with each other to potentially increase fibroblast cells amount within socket post tooth extraction of male wistar mouse.

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