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UNIVERSITAS AIRLANGGA FACULTY OF DENTAL MEDICINE
HEALTH RESEARCH ETHICAL CLEARANCE COMMISSION

ETHICAL CLEARANCE CERTIFICATE

Number : 304/HRECC.FODM/XII/2017

Universitas Airlangga Faculty Of Dental Medicine Health Research Ethical Clearance Commission has studied the proposed research design carefully, and therefore, shall herewith certify that the research entitled :

"EFEK PEMBERIAN EKSTRAK DAUN AVOKAD (*Persea americana* Mill.) TERHADAP JUMLAH SEL FIBROBLAS PADA SOKET PASCA PENCABUTAN GIGI TIKUS WISTAR"

Principal Researcher : **KARINA AWANIS ADLA**

Unit/Institution/Place of Research : Universitas Airlangga Surabaya

CERTIFIED TO BE ETHICALLY CLEARED

Surabaya, December 29th, 2017
Chairman.

Prof. M. Rudianto, Dr. drg. MS. Sp.Perio(K)
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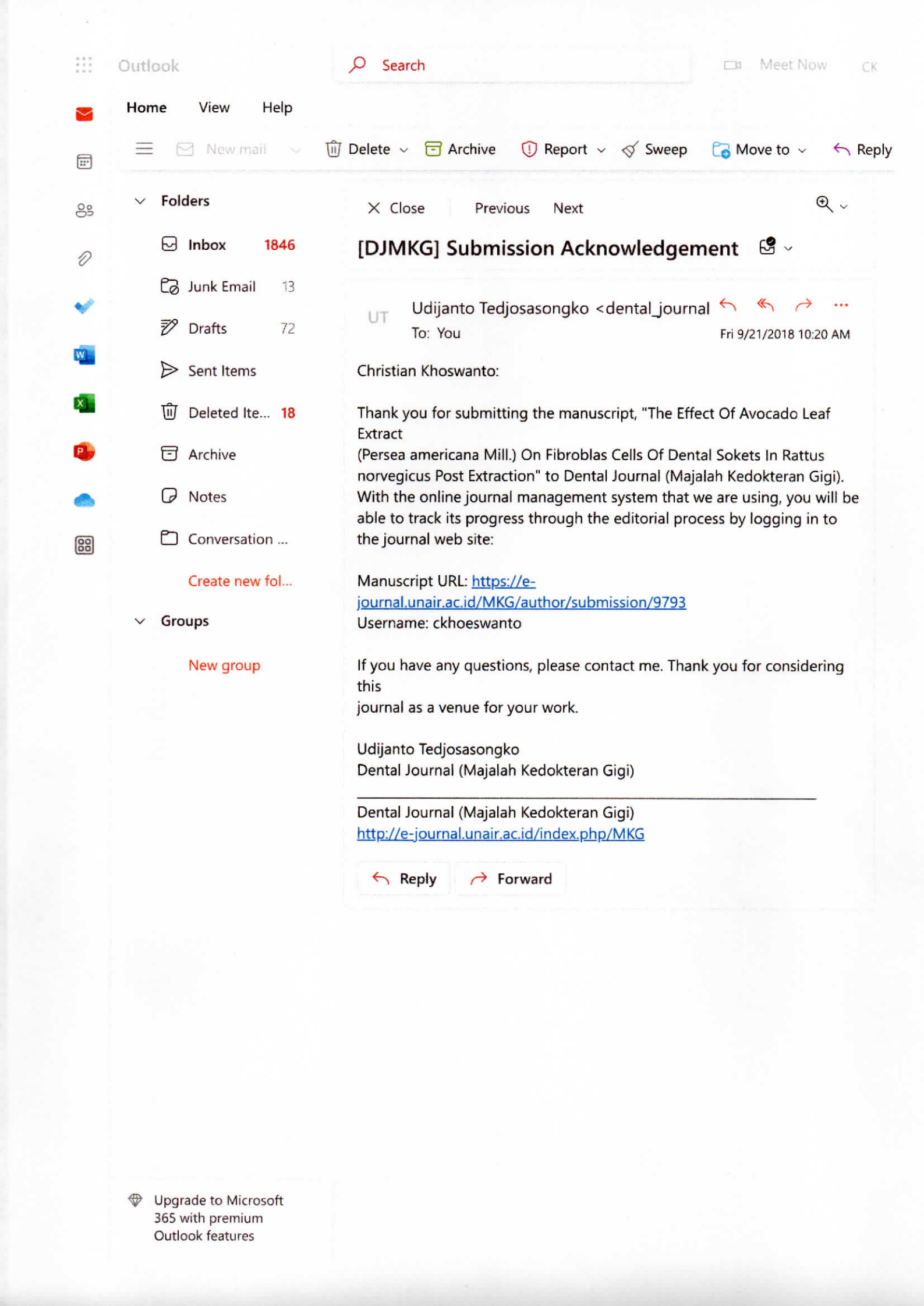
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The effect of avocado leaves extract (*Persea americana Mill.*) on fibroblast cells of dental sockets in wistar rats post tooth extraction

Reviewer 1 comments :

Abstract

- It is not clear meaning. T-TEST test obtained significance value 0,00 ($p<0.05$) Please rewrite
- Mana yang benar Acceleration or against?? Sebagai indicator inflamasi ??? Di paragraph terakhir introduction Spt ini: "The aim of this study was to understand the effect of Avocado leaves extract against Fibroblast proliferation rate and inflamation indicator"
- Mau menggunakan Post tooth extraction atau after tooth extraction??? Harus konsisten

Introduction

- Fix the sentence!
- Reduce bleeding time??
- Jangan pakai istilah "against" karena artinya melawan/atau hal yang bertentangan. Sementara anda bermaksud menstimulasi proliferasikan? Kalau Against inflammation benar
-

Material & Methods

- Is this term usually used??? Or maybe used "mandibular jaw taken..."
- What preparation ?? please make it detail i.e decalsification with ... than tissue processing done using paraffin embedded-tissue method.... Tissue cuted using microtome with... in thickness amd lastly stainng with....
- Make it material and methods directly in several paragraph without sub title (simple and clear) Please explain Lokasi pembacaan HPA pada soket gigi di daerah mana??, berapa org yang membacanya, pengambilan data pada berapa lokasi!!
- Dikeringkan dengan menggunakan blender?
- Mandibular retrieve kemudian ada lagi istilah decapitation mandible??? Ini bedanya apa?? gunakan kata yang konsisten Kemudian apakah anda pakai istilah mandibular atau mandible?
- Siapa yang mengamati dan berapa orang yang mengamati, bagaimana cara menghitungnya?? Berapa area??? Jelaskan
- Gambar HPA harus diambil di dalam soket gigi U tunjukkan gambar soket giginya dulu dengan pembesaran 100x. harusnya akar gigi Insisivus centralis kanan sebagai batas mesial dinding soket insisvus kiri kelihatan
-

Result

- What this (CGID3,CG2D7).....stand for? Please explain in the text
- Fibroblast morphology??shown in control group. Light microscope (400X magn)
- Better put the figures (control and treatment) in one panel. Each for 3 and 7 days results. Most importantly, write a comparation needed to compare between the groups (morphology or number) . Do not leaf the figure w/o any explantion
- First ..Shows tooth socket completely with low magnification ◊ put in the left side then show fibroblast cells with high magnification in the right side. Make it in one picture panel for all group on day 3 and other picture panel for day 7

Discussion

- Please explain, your study focused on fibroblast proliferation and you didn't explore other factors involve in such healing process.
- These has been explained in Material & Methods, do not repeat
- Do not repeat the Material & Methods. Just focus on the results
- Did you observed all these (yellow hilight)? Otherwise, please explain, "In this study, you only focused the observation on.....
- You didn't observe this. Otherwise, please add references needed
- If you do not compare this reference to your result, it would better to delete this part. The discussion session should be used to explain your data, not necessary to explain literatures review
- You need to put more than one reference in this long quote
- If you do not compare this reference to your result, it would better to delete this part. The discussion session should be used to explain your data, not necessary to explain literatures review
- What do you mean? Pls focuse on the data you go
- I do not see the data???. How do you know the tested material do not cause toxic effect??
- You need to explain this important statement. Your data you got only showed the effect in proliferation, not its capability in accelerating the fibroblast grew in the presence of the tested material.
- First, shows your data and discussing your data using this reference
- Bagaimana hubungan proliferasi fibroblast dengan flavonoid? Kenapa peningkatan proliferasi fibroblast dihubungkan dengan anti inflammatory dari flavonoid?
- Mengapa kok bicara antioksidan ? apakah pada pencabutan gigi banyak radikal bebas?
- Kalau di dalam socket gigi apakah juga terbentuk myofibroblast bukankah ini terjadi pada soft tissue healing? Apakah ada hubungannya dengan reepitelisasi untuk penutupan socket gigi

Reviewer 2 comments :

Abstract

- Fixed the sentence!!! Tooth extraxtion does not cause to blood vessel in wound healing process but.. it makes trauma to blood vessel in alveolar bone of tooth socket
- Check the meaning of decapitate →caput = mean "head"
- The preposition using " in" or "on"..?????

Material & Methods

- Decaputation artinya pemotongan kepala atau pematahan tulang leher kepala tikus yaitu merupakan salah satu cara pembunuhan hewan coba. Cara lain adalah euthanasia. Setelah hewan coba didekaputasi atau di euthanasia◊ mandibula di ambil (mandibular jaw taken….ect

REFERENCES

- : Nama jurnal di cetak miring tapi yang lainnya tidak

Surabaya, 24 Maret 2023

Mengetahui

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Ketua Editor Dental Journal
Periode tahun 2013 - 2018

Udijanto Tedjosasongko, drg.,Ph.D., Sp.KGA(K)

Research Report

The effect of Avocado leaves extract (Persea americana Mill.) on fibroblast cells of dental sockets in wistar rats post tooth extraction

ABSTRACT

***Background**: In the dental profession, tooth extraction is a common practice. Tooth extraction action causes trauma to blood vessels in wound healing process. Acceleration of wound healing, where Fibroblasts play an important role is influenced by nutrition. Avocado leaves contain a variety of chemicals, including flavonoid compounds, tannins, katekat, kuinon, saponin, and steroids / triterpenoid. Avocado leaves also contain glycosides, cyanogenic, alkaloids and phenols function as anti-inflammatory, antibacterial, and antioxidant. With these contenst of the avocado leaves could be used as an alternative medicine to accelerate wound healing process in socket post tooth extraction. **Purpose**: To determine the effect of avocado leaves (Persea americana Mill) in acceleration of fibroblast cells proliferation in socket after tooth extraction. **Methods**: 24 Wistar rats were divided into two groups of controls on the third and seventh day and two groups of avocado leavesextract gels with the third and the seventh maximum concentrations. The gel was applied to the extraction socket of the lower left mandibular incisor. After the third and seventh day, a mandibular decaputate and a tooth extraction socket were prepared by HPA (Histology Pathology Anatomy) with Hematoxylin Eosin (HE) staining. The fibroblast proliferation was analyzed by light microscope with 400x magnified. The obtained data were analyzed using t-Test test.**Result**: t-Test obtained significance value 0,001 (p <0,05) between control and treatment group. The number of fibroblast cells increased in the third day treatment group, and decreased in the treatment group on the seventh day. **Conclusion**: Avocado leaves extract (Persea americana Mill.) accelerates proliferation of fibroblast cells in tooth socket wistar rats post tooth extraction.*

***Key words:** avocado leaves extract, wound healing, fibroblast*

Commented [S1]: Mana yang benar Acceleration or against?? Sebagai indicator inflamasi ???
Di paragraph terakhir introduction Spt ini:
"The aim of this study was to understand the effect of Avocado leaves extract against Fibroblast proliferation rate and **inflamation indicator**"

Commented [S2]: Mau menggunakan Post tooth extraction atau after tooth extraction??? Harus konsisten!!!

INTRODUCTION

In the dental profession, tooth extraction is one of the most common procedure done in practice. Tooth extraction may cause trauma to the blood vessels. After trauma occurs to the blood vessels, the hemostasis process begins. Hemostasis is a process of blood clotting on the walls of damaged blood vessels to stop bleeding. The process of wound healing post-dental extraction can sometimes cause infections and may even lead to complications. Patients require proper handling

post-extraction to reduce the possibility of complications, as well as to accelerate blood clotting thus making the process of wound healing after tooth extraction faster.[5]

The wound healing process itself is quite complex, consisting of various processes and is assisted by many cells, one of them are fibroblasts. Fibroblasts are cells in connective tissue. Fibroblasts are responsible for phagocytosis of bacteria.[10] TGF-β (*transforming growth factor β*) and PDGF (*platelet- derived growth factor)* stimulate fibroblasts structure to miofibroblasts located at the edges of ECM to help wound closure of the tissues.[10]The fibroblasts will appear in the wound area after 3 days and the number of fibroblast cells will peak on the 7th day after trauma.[6]

Avocados are a type of the plants that have the benefits of traditional medicine.[9] Almost all parts of the avocado plants have properties akin to a source of medicine. The parts of an avocado has many benefits;the leaves, fruit and also the seeds have a high nutrient content. Avocado leaves contain a variety of chemicals, including flavonoid compounds, tannins, katekat, kuinon, saponin, and steroids/triterpenoid. Avocado leaves also contain glycosides, cyanogenic, alkaloids and phenols.[9]

The aim of this study was to understand the effect of Avocado leaves extract against Fibroblast proliferation rate and inflamation indicator.

Commented [S3]: Jangan pakai istilah "against" karena artinya melawan/atau hal yang bertentangan. Sementara anda bermaksud menstimulasi proliferasikan? Kalau Against inflammation benar!

MATERIALS AND METHODS

In this study, we used a rat model to evaluate wound healing activity indicated fibroblast proliferation. The approval of the ethical board has been taken (304/ HRECC.FODM/XII/2017).

This study used post-test only control group design with 24 samples of male Wistar rats, 150-200 gr in weight, 2-3 months of age and adaptated for 1 week with pellet food and standard ad libitiumaquades.

The sample was divided into 4 groups, control groups (n= 6) and thetreatment groups (n= 6). In the control group, Wistar rats were given a 3% CMC Na solution to synchronize the physiological state of the Wistar rat's body; the 3% CMC Na solution did not have a negative effect on tissue or animal organ,[16]while the treatment group was given avocado leaves extract and 3% CMC Na solution as 0.1 cc solvent on the Wistar rat's tooth socket. Both control and treatment group had ~~its~~ mandibular jaw taken on the 3rd and 7th day and ~~decapitated and~~ all specimens ~~needed~~ were prepared ~~within the experiment period~~ for tissue processes.

Avocado leaves were obtained and identified at UPT MateriaMedica, Kota Batu, East Java. Fresh Avocado leaves ~~are~~ washed thoroughly, dried and mashed using a blender with 96% ethanol solvent, then put into a jar with a tightly closed for 24 hours, shake it on a digital shaker at 50 rpm.

Commented [S4]: Dikeringkan dengan menggunakan blender??

The obtained liquid extract were filtered using a cloth filter??, then put in Erlenmeyer tube. Further the liquid extract was evaporated with a rotary evaporator for 1 hour and 30 minutes. The resulting extract was evaporated over the waterbath for 2 hours and stored in freezer until used.

General anesthesia was performed on Wistar rats using chloroform inhalation. Tooth extraction was performed on the left mandibular incisive on using pliers. After the extraction, irrigation is done using sterile aquades to clean up the rest of the debris. To stop the post-extraction bleeding, a sterile cotton roll was pressed into the tooth extraction socket.

The treatment protocol was performed acording to Krinke as follow. After the removal and discontinuation of bleeding on Wistar rat's tooth sockets, the Wistar rats were treated. ~~The sample was divided into 4 groups control groups (n= 6) and the treatment groups (n= 6).~~

The treatment group was chosen to have its mandibula decaputated and made into preparations on the 3rd and 7th day. The decaputation of the mandible in the treatment group and the preparation of the 3rd and 7th day were done because fibroblasts appeared on the wound area 3 days after the trauma and then peaks after 7 days.[12]On the 3rd and 7th day, a mandibular retrieval procedure was performed by attempting to anesthesize the Wistar rats with a lethal dose of chloroform. Anesthesia was performed by putting Wistar rats into a glass gas chamber which was filled with 10% chloroform until the wistar rats are a sleep.The wistar rats in each group had their mandibula decaputated and was then buried properly. Decaputated Mandibles are made into tissue preparation, and then stained with HE (HaematoxylinEeosin) and observed. Histopathologic observation was done by counting the number of fibroblasts under a light microscope with 400x magnification.

Commented [S5]: Perbaiki kalimatnya

Commented [S6]: Sudah disebut diatas tdk uusah diulang lagi

Commented [S7]: Apa maksudnya???

Commented [S8]: Perbaiki kalimat nya

Commented [S9]: Perbaiki kalimatnya

Commented [S10]: Mandibular retrieve kemudian ada lagi istilah decapitation mandible??? Ini bedanya apa?? gunakan kata yang konsisten
Kemudian apakah anda pakai istilah mandibular atau mandible?

Commented [S11]: Perbaiki kalimat!!

Commented [S12]: Siapa yang mengamati dan berapa orang yang mengamati, bagaimana cara menghitungnya?? Berapa area??? jelaskan

RESULTS

The results showed (table. 1) after 3 day experimental period the number of fibroblasts in treatment group increased compared tocontrol group. Conversely, after 7 day the number of fibroblast cells in treatment group was less than the control group.

Table 1. Mean amount of fibroblast in treatment group and control group

Group	X±SD Day 3	X±SD Day 7
K1	3.00^{b}±0.89	14.33^{b}±0.51
K2	20.33^{a}±1.75	10.16^{b}±0.75

note : different superscript showed significance diference ($\alpha < 0.05$)
K1 : Control group
K2 : Treatment group

Table 1.shows the number of fibroblast proliferation on day 3 shows there is a significant difference in the wound between treatment group and control group.While on day 7 did not shows significant difference between treatment group and control group.

Figure 1: FibroblastsHPA in control group; 3rd day (400x magnification)

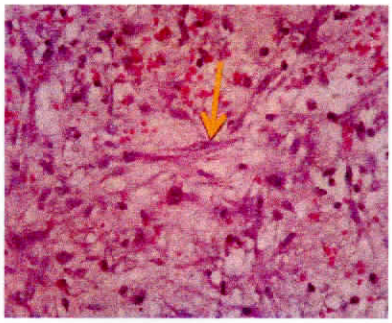
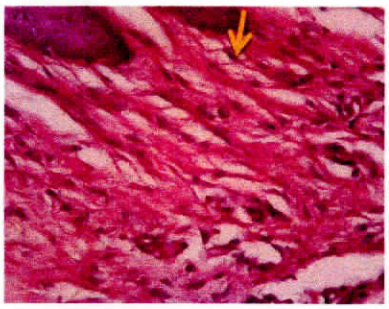


Figure 2: Fibroblasts after the application of avocado leaves extract for 3 days (400x magnification)



Commented [S13]: Gambar HPA harus diambil di dalam soket gigi → tunjukkan gambar soket giginya dulu dengan pembesaran 100x. harusnya akar gigi Insisivus centralis kanan sebagai batas mesial dinding soket insisvus kiri kelihatan!!

Commented [S14]: Gambar ini di copy paste dengan hari ke -7 → perbaiki???

Figure 3: Fibroblasts from the control group; 7th day (400x magnification)

Commented [S15]: Gambar HPA fibroblast hari ke 7 sama dengan gari ke -31!! kenapa sama Perbaiki!!

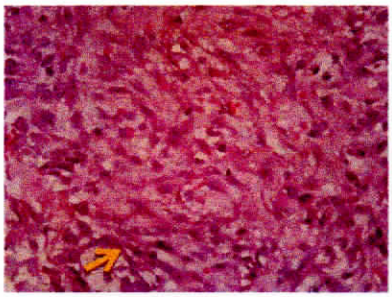


Figure 4: Fibroblasts after the application of avocado leaves extract for 7 days (400x magnification)

DISCUSSION

The process of tooth extraction will leave a wound. After, there will be a healing process through a series of complex processes involving a number of cells, cytokines, growth factors, and extracellular components that play a role in repairing damage to the hard tissue and soft tissue.[2]

Commented [S16]: Mohon perhatikan penggunaan "a"

The wound healing process is influenced by several factors including bacterial infections, damage to the tissue, necrosis, hematoma (tissue bleeding), excessive movement of injured tissue, low blood supply and drug administration.[8]

The injured tissue gets an acute inflammatory reaction quickly. The inflammatory phase prepares areas for healing and wound immobilization. The acute inflammatory phase occurs instantaneously, characterized by the exudation of plasma proteins and neutrophils. The chronic inflammatory phase is characterized by chronic inflammatory cells (macrophages, lymphocytes, and plasma cells).[7] In this study we observed that the proliferation phase begins on the 3rd day and can last up to several weeks. [2]In proliferation phase neutrophil cells digest bacteria, then release intracellular enzymes into the surrounding matrix, then neutrophil cells die. Monocytes will come out of the blood capillaries into the ECM and turn into macrophages which are then mediated by the inflammatory mediator TGF β. TGF β activates fibroblast cells[10] and stimulates collagen deposition by increasing collagen synthesis. With the synthesis of collagen by fibroblasts, the formation of the dermis layer will be enhanced by regulating the balance between granulation tissue and dermis. So that the dermal epithelium and collagen layer have been formed.[2]

Commented [S17]: Kok bicara dermis?? Kenapa tidak epitel oral mukosa???

Acceleration of wound healing can be seen from several indicators, one of which is the number of fibroblasts.[2]Fibroblasts is key to the proliferative phase of wound healing, such as destroying fibrin clot, forming collagen, elastin, glycosaminoglycan, and proteoglycans induced by TGF-β to form a new extracellular matrix to close the wound and affect the process of reepitelization of the wound.[10] Thus, as indicated in this study the more fibroblasts appears on the socket sample, the faster the wound healing process might occurs.

In this study, on the 3rd day there was an increase in the number of fibroblast cells due to the active substances contained in the avocado leaves (*Perseaamericana* Mill) such as flavonoids that act as an anti-inflammatory through inhibition of cyclooxygenase and lipoxygenase, thus able to limit the number of inflammatory cells that migrate to the wound area. Flavonoids play an important role in maintaining permeability and increase capillary vascular resistance, therefore flavonoids are used in pathological conditions such as disruption of permeability of blood vessel walls.Flavonoids and phenol substances in avocado leaves accelerate wound healing through the mechanism of antioxidants in inhibiting the activity of free radicals by donating hydrogen atoms and bonding to unstable free radicals that can cause damage to cell membranes so that cells do not function perfectly. The existence of this bond will make free radicals become more stable, so that damage to cell membranes can be reduced and the proliferation phase occurs more quickly. This causes a shorter inflammatory reaction and TGF-β proliferation occurs earlier, and produces fibroblasts. In addition, avocado leaves also contain tannins, which is an active substance, increase the formation of fibroblast cells and capillary blood vessels, causing growth factor to stimulate the proliferation of fibroblast cells[1,13,17]

Other content in avocado leaves (*Perseaamericana* Mill) such as saponins, which is an active substance, also increases monocyte proliferation which can increase the number of macrophages that will secrete growth factors such as EGF, FGF, PDGF, and TGF-β that can stimulate migration and proliferation of fibroblasts into wound area,to more rapidly to synthesize collagen. [1,15]

In this study, the 7th day showed decrease in the number of fibroblast cells. Due to fibroblast cell production on day 3 was significant increased, fibroblasts are sufficient to synthesize collagen early so that on day 7 fibroblast cell observation the number of fibroblast cells decreases as fibroblasts cells are transformed into miofibroblasts located on the margins of ECM edge of wound tissue closure.[10]

Commented [S18]: Bagaimana hubungan proliferasi fibroblast dengan flavonoid? Kenapa peningkatan proliferasi fibroblast dihubungkan dengan anti inflammatory dari flavonoid?

Commented [S19]: Mengapa kok bicara antioksidan ? apakah pada pencabutan gigi banyak radikal bebas??

Commented [S20]: Kalau di dalam socket gigi apakah juga terbentuk myofibroblast→ bukankah ini terjadi pada soft tissue healing? Apakah ada hunhubungannya dengan reepitelisasi untuk penutupan socket gigi

This study showed avocado leaves (*Perseaamericana* Mill) that topically added into the post-extraction socket was capable of accelerating the amount of fibroblast on wound healing process in the Wistar rat's tooth socket.

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Research Report

The Effect Of Avocado Leaf Extract (Persea americana Mill.) On Fibroblas Cells Of Dental Sokets In Rattus norvegicus Post Extraction

ABSTRACT

Background: *Tooth extraction is a common practicing in the dental profession, ~~tooth extraction is a common practice in practice~~. Tooth extraction action causes trauma to blood vessels ~~Fibroblasts play an important role~~ in wound healing process. Acceleration of wound healing, where fibroblasts play an important role, is influenced by nutrition. Avocado leaves contain a variety of chemicals, including flavonoid compounds, tannins, katekat, kuinon, saponin, and steroids / triterpenoid. Avocado leaves also contain glycosides, cyanogenic, alkaloids and phenols function as anti-inflammatory, antibacterial, and antioxidant. With these contents, ~~of the~~ avocado leaves ~~avocado~~ could be used ~~plays a role again~~ as an alternative medicine ~~st the~~to acceleration ~~the~~of wound healing process ~~on~~in socket ex-tooth extraction.* ***Purpose***: *To determine the effect of avocado leaves (Persea americana Mill) in acceleration ~~on the number~~ of fibroblast cells proliferation in socket after tooth extraction.* ***Methods***: *24 Wistar rats were divided into two groups of controls on the third and seventh day and two groups of avocado leaf extract gels with the third and the seventh maximum concentrations. The gel was applied to the extraction socket of the lower left mandibular incisor. After the third and seventh day, a mandibular decapitate and a tooth extraction socket were prepared by HPA with Hematoxylin Eosin (HE) staining. The ~~number of~~ fibroblast proliferation ~~cells~~ was analyzed by calculating ~~by observing~~ the cell number under light ~~a 400x magnification~~ microscope. ~~Research~~ The obtained data were analyzed using Student t ~~T-TEST~~ test and continued with LSD test.* ***Result***: *T-TEST test obtained significance value 0,00 (p <0,05) and LSD test obtained significant difference between control and treatment group. The number of fibroblast cells increased in the third day treatment group, and decreased in the treatment group on the seventh day.* ***Conclusion***: *Avocado leaf extract (Persea americana Mill.) ~~i~~Increased/accelerates proliferation ~~the number~~ of fibroblast cells ~~in~~ postoperative Wistar tooth extraction syringes.*

Key words: *avocado leafs extract, wound healing, fibroblast*

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INTRODUCTION

In the dental profession, tooth extraction is one of the most common procedure done in practice. Tooth extraction may cause trauma to the blood vessels. After trauma occurs to the blood vessels, the hemostasis process begins. Hemostasis is a process of blood clotting on the walls of damaged blood vessels to stop bleeding. The process of wound healing post-dental extraction can sometimes cause infections and may even lead to complications. Patients require proper handling post-extraction to reduce the possibility of complications, as well as to accelerate blood clotting thus making the process of wound healing after tooth extraction faster.[1]

The wound healing process itself is quite complex, consisting of various processes and is assisted by many cells, one of them are fibroblasts (reference?). Fibroblasts are cells in connective tissue ~~that are influential in the wound healing process~~ and ~~. Fibroblasts~~this cell ~~are~~is responsible for phagocytosis of bacteria (REFERENCE?), ~~and fibroblasts will undergo some phenotypic changes~~. TGF-β and PDGF (write the full name first) stimulate fibroblasts structure to miofibroblasts located at the edges of ECM to help wound closure of the tissues.[2] The fibroblasts will appear in the wound area after 3 days and the number of fibroblast cells will peak on the 7th day after trauma.[3]

Avocados are a type of the plants that have the benefits of traditional medicine (Reference). Almost all parts of the avocado plants have properties akin to a source of medicine. The parts of an avocado has many benefits; the leaves, fruit and also the seeds have a high nutrient content. Avocado leaves contain a variety of chemicals, including flavonoid compounds, tannins, katekat, kuinon, saponin, and steroids/triterpenoid. Avocado leaves also contain glycosides, cyanogenic, alkaloids and phenols.[4]

The aim of this study was…….. Fibroblst proliferation rate and inflammation indicator????

MATERIALS AND METHODS

In this study, we used a rat model to evaluate wound healing activity of….. The approval of the ethical board has been taken (number of approval of the ethics ……..)

~~The type of research conducted ,is a laboratory experimental research, which is done with intervention in providing treatment to the object of research~~. To do this, we ~~The design of this study~~ used post-test only control group design with 24 samples of male W~~w~~istar rats, 150-200 gr in weight, 2-3 months of age and adapt~~a~~ted for 1 week with pellet food and standard ad libitium aquades.

The sample was divided into 4 groups, i.e ~~2~~ the control group~~s~~ (n = 2)and ~~2~~ the treatment group~~s~~ (n= 2). In the control group, Wistar rats were given a 3% CMC Na solution to synchronize the physiological state of the Wistar rat's body; the 3% CMC Na solution did not have a negative effect on tissue or animal organ (add reference). ~~W~~while the treatment group was given avocado leaf extract and 3% CMC Na solution as 0.1 cc solvent on the Wistar rat's tooth socket. The treatment group was chosen to have its mandibula decaputated and all speciments needed were ~~made into~~ prepare~~d~~ations on the 3rd and 7th day……of experiment periods.

~~PRODUCTION~~ PreparationOF AVOCADO LEAF EXTRACT

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Avocado leaves were obtained and identified at UPT Materia Medica, Kota Batu, East Java. Fresh Avocado leaves are washed thoroughly, dried and mashed using a blender with 96% ethanol solvent, put into a jar with a tightly closed for 24 hours. Then shake it on a digital shaker at 50 rpm. The obtained liquid extract ~~is~~ were filtered using~~with~~ a cloth filter, then put ~~it is accommodated~~ in an Erlenmeyer tube. Further, ~~T~~the liquid extract was (were??) evaporated with a rotary evaporator for 1 hour and 30 minutes. The resulting extract was (??) evaporated over the water-bath for 2 hours, and ~~. After that the existing liquid extract is~~ stored in ~~the container~~ freezer.....or left in room temperaur until used.

TREATMENT OF ANIMALS

General anesthesia was performed on Wistar rats using chloroform inhalation. Tooth extraction was then performed on the left mandibular incisive on using pliers. After the extraction, irrigation was ~~is~~ done using sterile aquades to clean up the rest of the debris. To stop the post-extraction bleeding, a sterile cotton roll??was pressed into the tooth extraction socket.

The treatment protocol was performed according to.....(reference??), as follow: After the removal and discontinuation of bleeding ~~on Wistar rat's tooth sockets~~, the Wistar rats were treated. The sample was divided into 4 groups, ~~ie :~~ ~~2~~ control group~~s~~ (n =2) and ~~2~~ treatment group~~s~~ (n = 2).

Wistar rats were given a 3% CMC Na solution to synchronize the physiological state of the Wistar rat's body; the 3% CMC Na solution did not have a negative effect on tissue or animal organ. While the treatment group was given avocado leaf extract and 3% CMC Na solution as 0.1 cc solvent on the Wistar rat's tooth socket. The treatment group was chosen to have its mandibula decaputated and made into preparations on the 3rd and 7th day. The decaputation of the mandible in the treatment group and the preparation of the 3rd and 7th day were done because fibroblasts appeared on the wound area 3 days after the trauma and then peaks after 7 days.[5]

On the 3rd and 7th day, a mandibular retrieval procedure was performed by attempting to anesthesize the Wistar rats with a lethal dose of chloroform. Anesthesia was performed by putting Wistar rats into a glass gas chamber which was filled with 10% chloroform until the wistar rats are asleep.

The wistar rats in each group had their mandibula decaputated and was then buried properly. Decaputated Mandibles are made into tissue preparation, and then stained with HE (Haematoxylin Eeosin) and observed. Histopathologic observation was done by counting the number of fibroblasts under a light microscope with 400x magnification.

RESULTS

The results showed (diagram 1), after 3 day experimental period, the number of fibroblasts ~~on the 3rd day~~ in treatment group was increased compared to those in ~~the 3rd day~~ in control group. Conversely, after 7 day, ~~While~~ the number of fibroblast cells ~~on the 7th day~~ in treatment group was less than the control group ~~on day 7~~. ~~The average diagram of the number of fibroblasts per group can be seen in diagram 1.~~

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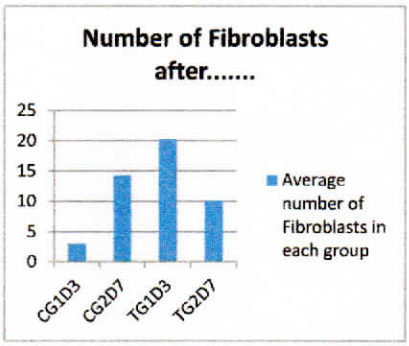

Diagram 1: Average number of Fibroblasts in each group

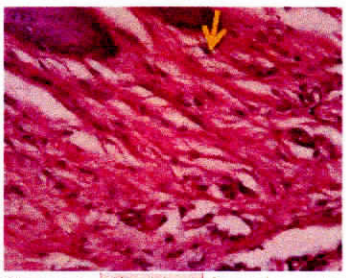

Figure 1: Fibroblasts from the control group; 3rd day (400x magnification)

Commented [ARH3]: Fibroblast morphology??shown in control group. Light microscope (400X magn)

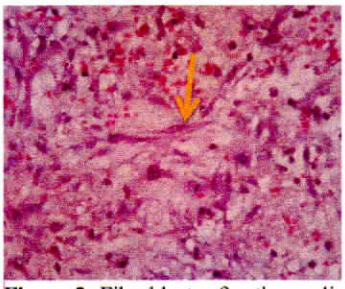

Figure 2: Fibroblasts after the application of avocado leaf extract for 3 days (400x magnification)

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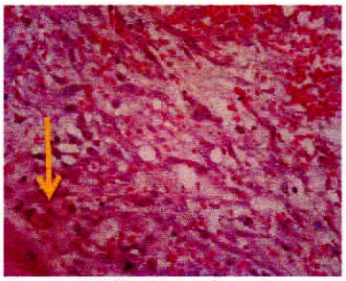

Figure 3: Fibroblasts from the control group; 7th day (400x magnification)

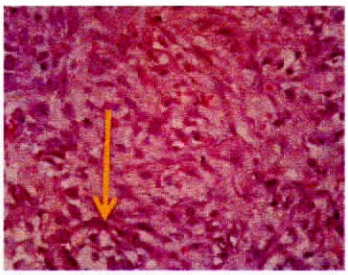

Figure 4: Fibroblasts after the application of avocado leaf extract for 7 days (400x magnification)

DISCUSSION

The process of tooth extraction will leave a wound. After,??? there will be a healing process through a series of complex processes involving a number of cells, cytokines, growth factors, and extracellular components that play a role in repairing damage to the hard ~~tissue~~ and soft tissues.[6]

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The wound healing process is influenced by several factors including bacterial infections, caused by bacteria such as the *streptococcus* bacteria, damage to the tissue, necrosis, hematoma (tissue bleeding), excessive movement of injured tissue, low blood supply and drug administration. ~~In this study, the post-extraction tooth socket in the control group was applied with the carrier substance ie CMC Na gel and the treatment group was applied with an avocado leaf extract (*Persea americana* Mill) with maximum concentration.~~ ~~The wound healing process is then observed on the 3rd day and the 7th day; on the 3rd day and the 7th day, the wound healing process is in the inflammatory phase and the proliferation phase.~~

The injured tissue gets an acute inflammatory reaction quickly. The inflammatory phase prepares areas for healing and wound immobilization. The acute inflammatory phase occurs instantaneously, characterized by the exudation of plasma proteins and neutrophils. The chronic inflammatory phase is characterized by chronic inflammatory cells (macrophages, lymphocytes, and plasma cells).[7] In the inflammatory phase, macrophages play an active role in initiating the development of granulation tissue and release pro-inflammatory cytokines such as IL-1 and IL-6 and growth factors such as EGF, FGF, TGF-α, TGF-β, and PDGF Growth factor PDGF and TGF-β play a role in stimulating migration and proliferation of fibroblasts, blood vessels, and epithelium marking the proliferative phase.[8]

TGF-β1 is an inflammatory regulator of the wound. TGF-β has the role of enhancing extracellular matrix (ECM) and increasing collagenation, so that if TGF-β decreases, the cytokine that has the role of wound healing decreases, thus causing deceleration of wound healing. Extracellular matrix also plays an active role in wound healing by interacting with cells through a receptor called integrin, leading to platelet activation, migratory epithelial and fibroblast movements.[9] TGF-β stimulates the formation of fibroblast cells.[10]

~~After the inflammatory phase is over~~In this study, we observed that the proliferation phase begins on the 3rd day and can last up to several weeks.[6] Acceleration of wound healing can be seen from several indicators, one of which is the number of fibroblasts and TGF-β cells (??). As shown in Fig….., ~~T~~the structure-forming cells are fibroblasts, which secrete collagen frameworks (REFERENCE). Fibroblasts are cells in connective tissue that affect the wound healing process (REFERENCE). Fibroblasts will undergo some phenotypic changes and TGF-β and PDGF stimulate fibroblasts structure into miofibroblasts located at the edges of ECM to help wound closure of the tissue.[2] Fibroblasts and TGF-β are important components in supporting wound healing as they are involved in multiple processes which is key to the proliferative phase of wound healing, such as destroying fibrin clot, forming collagen, elastin, glycosaminoglycan, and proteoglycans induced by

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TGF-β to form a new extracellular matrix to close the wound and affect the process of reepitelization of the wound.[2] Thus, as indicated in this study, the more fibroblasts ~~that~~ appears on the socket sample, the faster the wound healing process might occurs.

In this study, on the 3rd day there was an increase in the number of fibroblast cells due to the active substances contained in the leaf avocado (*Persea americana* Mill). We assumed the active substance could be ~~such as~~ flavonoids, as this in organic biomaterial ~~that~~ acts as an anti-inflammatory through inhibition of cyclooxygenase and lipoxygenase (REFERENCE), ~~,~~ thus it may involve ~~able~~ in ~~to limit~~restricing the number of inflammatory cells that migrate to the wound area (REFERENCE). This causes the inflammatory reaction to be shorter and TGF-β proliferation occurs earlier, so TGF-β works faster by producing (???) fibroblasts to synthesize collagen (REFERENCE/s). Flavonoids also accelerate wound healing through the mechanism of antioxidants inhibiting free radical activity.[12] YOU NEED TO EXPLAIN THE ASSOCIATION BETWEEN WHAT YOU HAD FOUND (THE STUDY RESULT) AND ALL OF THESE REFERENCE QUOTED

Other content in avocado leaves (*Persea americana* Mill) such as saponins, which is an active substance, also increases monocyte proliferation which can increase the number of macrophages that will secrete growth factors such as EGF, FGF, PDGF, and TGF-β that can stimulate migration and proliferation of fibroblasts into wound area, synthesize collagen, and increase the proliferation of capillary blood vessels.[13,14]

~~The phenol on avocado leaves act as antioxidants by neutralizing free radicals to accelerate wound healing. In addition, avocado leaves also contain tannins which also increase the formation of fibroblast cells and capillary blood vessels, causing growth factor to stimulate the proliferation of fibroblast cells.[11]~~

In this study, the 7th day showed a decrease in the number of fibroblast cells after the removal of avocado leaves (*Persea americana* Mill) at maximum concentration. Due to increased fibroblast cell production on day 3, fibroblasts are sufficient to synthesize collagen early so that on day 7 fibroblast cell observation the number of fibroblast cells decreases as fibroblasts cells are transformed into miofibroblasts located on the margins of ECM edge of wound tissue closure.[2]

In conclusion: This study proves that avocado leaves (*Persea americana* Mill) have many benefits. The avocado leaf extract (*Persea americana* Mill) ~~given~~ that topically added into the post-extraction tooth socket proved to be non-toxic and capable of accelerating the wound healing process in the Wistar rat's tooth socket after tooth extraction by increasing the number of fibroblast cells.

REFERENCES

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Research Report

Revised

The effect of Avocado leaf extract *(Persea americana Mill.)* on the fibroblast cells of post-extraction dental sockets in Wistar rats

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ABSTRACT

Background: *Tooth extraction, a common practice among the dental profession, causes trauma to the blood vessels during the wound healing process. The acceleration of wound healing, within which fibroblasts play an important role, is influenced by nutrition. Avocado leaves contain a variety of chemicals, including flavonoid compounds, tannins, katekat, kuinon, saponin and steroids/triterpenoid. Avocado leaves also contain glycosides, cyanogenic, alkaloids and phenols which function as anti-inflammatory, antibacterial and antioxidant agents. This avocado leaf content could be used as an alternative medicine to accelerate the wound healing process in post-tooth extraction sockets.* ***Purpose:*** *To determine the role of avocado leaves (Persea americana Mill) in accelerating fibroblast cells proliferation in tooth socket post-extraction.* ***Methods:*** *The sample was divided into four groups, a control group and the treatment groups. The treatment groups used avocado leaf extract and 3% CMC Na solution and inserted into wistar tooth sockets. Both the control and treatment groups had their mandibula decapitated with all the required specimens being prepared on the 3rd and 7th days of the experiment period. Mandibular decapitation and tooth extraction socket were prepared by HPA (Histology Pathology Anatomy) with Hematoxylin Eosin (HE) staining. The fibroblast proliferation was analyzed by means of a light microscope at 400x magnification. The obtained data was analyzed using a t-Test.* ***Result:*** *The t-Test obtained a significance value 0.001 ($p < 0.05$) between the control and treatment groups. The number of fibroblast cells increased in the group treated on the third day and decreased in the group treated on the seventh day.* ***Conclusion:*** *Avocado leaf extract (Persea americana Mill.) accelerates proliferation of fibroblast cells in Wistar rats post-tooth extraction.*

Key words: *avocado leaf extract, wound healing, fibroblast*

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INTRODUCTION

Within the dental profession, one of the most common procedures performed is tooth extraction which may cause trauma to the blood vessels. After trauma occurs to the blood vessels, the hemostasis process, involving blood clotting on the walls of damaged blood vessels in order to prevent bleeding, commences. The process of post-extraction wound healing can occasionally cause infections, possibly even leading to complications.[1–4] Patients require appropriate post-extraction management in order to reduce the possibility of complications and accelerate blood clotting, thereby promoting wound healing after extraction. The wound healing process itself is relatively complex, consisting of various processes and assisted by many cells, one of them being fibroblasts. Fibroblasts are cells found in connective tissue responsible for the phagocytosis of bacteria. TGF-β (*transforming growth factor β*) and PDGF (*platelet- derived growth factor)* stimulate fibroblast structures to become miofibroblasts located at the edges of ECM which promote wound closure in tissues. Fibroblasts will appear in the wound area after three days with the number of fibroblast cells peaking on the seventh day after trauma.[5–8]

The avocado plant possesses the benefits of traditional remedies[9] since almost all its constituent parts possess properties akin to those of such medicines. The leaves, fruit and seeds all have a high nutrient content. Avocado leaves contain a variety of chemicals, including: flavonoid compounds, tannins, katekat, kuinon, saponin, steroids/triterpenoids, glycosides, cyanogenic compounds, alkaloids and phenols.[7–9]

The aim of this study was to determine the effect of avocado leaf extract on fibroblast proliferation rates and inflamation indicators.

MATERIALS AND METHODS

This study used rodent subjects to evaluate wound healing activity indicated by fibroblast proliferation. Approval by the ethical board was granted (304/ HRECC.FODM/XII/2017). This study used a post-test only control group design with 24 male Wistar rat subjects, 150-200 grams in weight and aged 2-3 months which were allowed to freely consume pellet food for one week.

The sample was divided into four groups, a control group (n=6) and the treatment groups (n=6). In the control group, the subjects were given a 3% CMC Na solution to synchronize the physiological state of their bodies which had no negative effect on their tissues or organs. The treatment groups had avocado leaf extract and 3% CMC Na solution as a 0.1cc solvent inserted into their tooth sockets. Both the control and treatment groups had their mandibula decapitated with all the required specimens being prepared on the 3rd and 7th days of the experiment period.

Fresh avocado leaves were obtained from and identified at UPT Materia Medica, Kota Batu, East Java. The leaves were washed thoroughly, dried and liquified in a blender with 96% ethanol solvent, placed in a tightly sealed jar for 24 hours and agitated in a digital agitator at 50 rpm. The resulting liquid extract was filtered by being passed through a cloth, inserted in an Erlenmeyer tube and subsequently evaporated in a rotary evaporator for 90 minutes and stored in a freezer until required.

A general anesthetic was administered to the subjects by means of chloroform inhalation. Tooth extraction was performed on the left mandibular incisor using pliers after which irrigation was carried out using sterile aquades to remove the remaining debris. In order to stop post-extraction bleeding, a sterile cotton roll was applied to the resulting socket. The treatment protocol adopted was that advocated by Krinke whereby, following removal of the teeth and discontinuation of bleeding from the sockets, the subjects were treated.[10]

The treatment group was selected to have its mandibula decapitated and made into preparations on the 3rd and 7th days. Decapitation of the mandible in the treatment group and preparation on the 3rd and 7th day were performed because fibroblasts appeared in the wound area three days after the trauma before peaking after seven days. On the 3rd and 7th days, a mandibular retrieval procedure was performed by anesthetizing the subjects in a glass gas chamber filled with 10% chloroform. The members of each group had their mandibula decapitated and appropriately disposed of. The decapitated mandibles were made into tissue preparation, before being stained with HE (Haematoxylin Eeosin) and observed.

Histopathologic observation was performed by counting the number of fibroblasts under a light microscope at 400x magnification. Data was analyzed by means of a One-way Anova test with a 5% significance rate and subsequently with an LSD test to establish whether a significant difference existed.[11,12]

RESULTS

The results in Table 1 show that after a 3-day experimental period the number of fibroblasts in the treatment group had increased compared to that in the control group (Figures 1 & 2). Conversely, after seven days the number of fibroblast cells in the treatment group was lower than that in the control group (Figures 3 & 4).

Table 1 shows the extent of fibroblast proliferation on day 3 was significantly different in the wounds in the treatment group and the control group, while on day 7 no such significant difference was observed between the two groups.

DISCUSSION

Tooth extraction will result in a wound which then undergoes a healing process consisting of a series of complex processes involving a number of cells, cytokines, growth factors and extracellular components that play a role in repairing damage to the hard tissue and soft tissue.[2] The wound healing process is influenced by several factors including: bacterial infections, damage to the tissue, necrosis, hematoma (tissue bleeding), excessive movement of injured tissue, low blood supply and drug administration.[8,13,14] The injured tissue rapidly experiences an acute inflammatory reaction. The inflammatory phase precedes healing and wound immobilization. The instantaneous acute inflammatory phase is characterized by the exudation of plasma proteins and neutrophils. The chronic inflammatory phase is characterized by the presence of chronic inflammatory cells (macrophages, lymphocytes, and plasma cells).[7] In this study, it was observed that the proliferation phase begins on the third day and can last for several weeks.[2] In the proliferation phase, neutrophil cells digest bacteria, then release intracellular enzymes into the surrounding matrix before expiring. Monocytes will move from the blood capillaries into the ECM, transforming into macrophages which are then mediated by the inflammatory mediator TGF β. TGF β activates fibroblast cells and stimulates collagen deposition by increasing collagen synthesis. With the synthesis of collagen by fibroblasts, the

formation of the epithelial layer will be enhanced by regulating the balance between it and the granulation tissue.[10,15,16] As a result, the mucous epithelium and collagen layer will form.[2]

Acceleration of the wound healing process can be confirmed by the presence of several indicators, one of which is the number of fibroblasts. Fibroblasts are key to the proliferative phase of wound healing, such as destroying fibrin clot, forming collagen, elastin, glycosaminoglycan and proteoglycans induced by TGF-β to form a new extracellular matrix to close the wound and affect the reepithelization process in the wound.[10] Thus, as indicated in this study, the more fibroblasts appear in the socket sample, the more rapid the wound healing process might be.[2]

This study showed that on the third day an increase in the number of fibroblast cells occurred due to active substances such as flavonoids contained in the avocado leaves (*Persea americana* Mill) that have an anti-inflammatory effect through inhibition of cyclooxygenase and lipoxygenase. In this manner, they are able to limit the number of inflammatory cells that migrate to the wound area. Flavonoids play an important role in maintaining permeability and increasing capillary vascular resistance. Therefore, flavonoids are present in pathological conditions such as disruption to the permeability of the blood vessel walls. Flavonoids and phenol substances in avocado leaves accelerate wound healing through antioxidant mechanisms which inhibit the activity of free radicals to donate hydrogen atoms and bond to unstable free radicals that can cause damage to cell membranes and impede cell functioning. The existence of this bond will render free radicals more stable, thereby reducing damage to cell membranes and enabling the proliferation phase to proceed more rapidly. This reduces the duration of the inflammatory reaction, induces earlier TGF-β proliferation and results in the production of fibroblasts. In addition, avocado leaves also contain tannins which are active substances that increase the formation of fibroblast cells and capillary blood vessels, causing growth factor to stimulate the proliferation of fibroblast cells.[1,13,17]

Other content of avocado leaves (*Persea americana* Mill) includes saponin, another active substance, which increases monocyte proliferation and can augment the number of macrophages that will secrete growth factors such as EGF, FGF, PDGF and TGF-β. These, in turn, can stimulate the migration to and proliferation of fibroblasts in the wound area in order to more rapidly synthesize collagen.[1,15]

This study showed a decrease in the number of fibroblast cells on the seventh day. Due to a significant increase in fibroblast cell production on day 3, fibroblasts are sufficient to

synthesize collagen. This has the result that, on day 7, the number of fibroblast cells decreases as they are transformed into myofibroblasts located on the ECM margins of wound tissue closure.[10,18]

This study showed that avocado leaves (*Persea americana* Mill) topically applied to the post-extraction socket were capable of accelerating the amount of fibroblast present in the wound healing process in Wistar rat tooth sockets.

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Group	X±SD Day 3	X±SD Day 7
K1	3.00^{b}±0.89	14.33^{b}±0.51
K2	20.33^{a}±1.75	10.16^{b}±0.75

Note: Different superscript showed significance difference ($p < 0.001$)

K1: Control group
K2: Treatment group

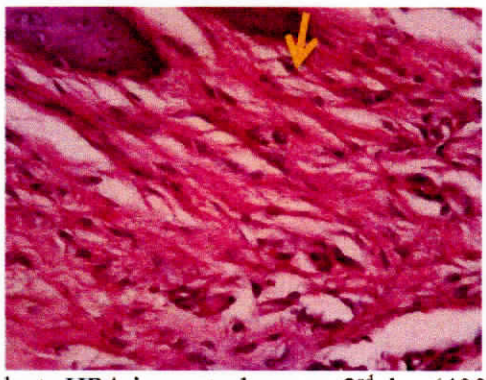

Figure 1. Fibroblasts HPA in control group; 3rd day (400x magnification)

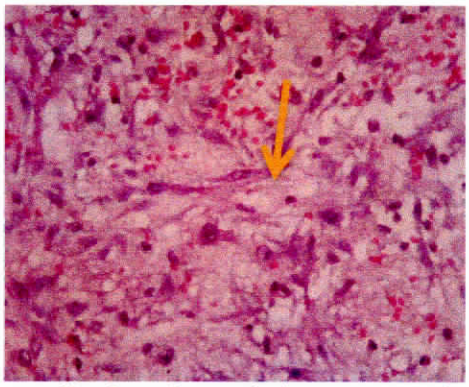

Figure 2. Fibroblasts after the application of avocado leaves extract for 3 days (400x magnification)

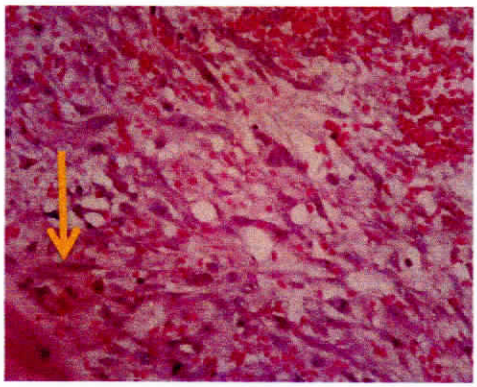

Figure 3. Fibroblasts from the control group; 7th day (400x magnification)

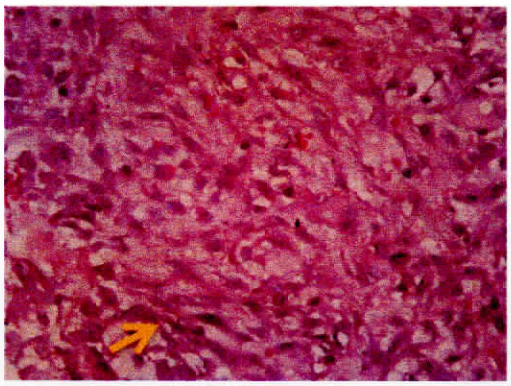

Figure 4. Fibroblasts after the application of avocado leaves extract for 7 days (400x magnification)

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Departemen Biologi Oral
Fakultas Kedokteran Gigi
Universitas Airlangga

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The effect of Avocado leaf extract (Persea americana Mill). on the fibroblast cells of post-extraction dental sockets in Wistar rats
Authors: Christian Khoswanto, Wisnu Setyari Juliastuti, and Karina Awanis Adla

Agar artikel tersebut dapat kami proses lebih lanjut untuk dapat diterbitkan pada Dental Journal (Majalah Kedokteran Gigi) Volume 51 Nomor 3 September 2018, penulis dimohon melakukan koreksi ulang sebelum artikel kami terbitkan. Hasil koreksi mohon dikirim kembali ke alamat E-mail: dental_journal@fkg.unair.ac.id dan dapat kami terima selambat-lambatnya tanggal **27 November 2018**. Apabila sampai batas waktu yang kami tentukan penulis tidak memberikan respon pada E-mail ini, maka kami anggap penulis telah menyetujui artikel tersebut untuk diterbitkan pada Dental Journal (Majalah Kedokteran Gigi) Volume 51 Nomor 3 September 2018.

Atas perhatiannya, kami ucapkan terima kasih.

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Volume 51, Number 3, September 2018

p-ISSN: 1978-3728
e-ISSN: 2442-9740

Dental Journal

Majalah Kedokteran Gigi

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Printed by: *Airlangga University Press*. (RK. 276/07.18/AUP-~~BHE~~). Kampus C Unair, Mulyorejo Surabaya 60115, Indonesia. Telp. (031) 5992246, 5992247, Telp./Fax. (031) 5992248. E-mail: aup.unair@gmail.com

Dental Journal
Majalah Kedokteran Gigi

Dental Journal
(Majalah Kedokteran Gigi)
2018 September; 51(3): 140–143

Research Report

The effect of Avocado leaf extract *(Persea americana Mill).* on the fibroblast cells of post-extraction dental sockets in Wistar rats

Christian Khoswanto, Wisnu Setyari Juliastuti, and Karina Awanis Adla
Department of Oral Biology,
Faculty of Dental Medicine, Universitas Airlangga
Surabaya – Indonesia

ABSTRACT

Background: *Tooth extraction, a common practice among the dental profession, causes trauma to the blood vessels during the wound healing process. The acceleration of wound healing, within which fibroblasts play an important role, is influenced by nutrition. Avocado leaves contain a variety of chemicals, including flavonoid compounds, tannins, katekat, kuinon, saponin and steroids/triterpenoid. Avocado leaves also contain glycosides, cyanogenic, alkaloids and phenols which function as anti-inflammatory, antibacterial and antioxidant agents. This avocado leaf content could be used as an alternative medicine to accelerate the wound healing process in post-tooth extraction sockets.* ***Purpose:*** *To determine the role of avocado leaves (Persea americana Mill) in accelerating fibroblast cells proliferation in tooth socket post-extraction.* ***Methods:*** *The sample was divided into four groups, a control group and three treatment groups. The treatment groups used avocado leaf extract and 3% CMC Na solution which was inserted into the tooth sockets of Wistar rats. Both the control and treatment groups had their mandibula decapitated with all the required specimens being prepared on the 3rd and 7th days of the experiment. Mandibular decapitation and tooth extraction socket were prepared by HPA (Histology Pathology Anatomy) with Hematoxylin Eosin (HE) staining. The fibroblast proliferation was analyzed by means of a light microscope at 400x magnification. The obtained data was analyzed using a t-Test.* ***Result:*** *The t-Test obtained a significance value 0.001 ($p < 0.05$) between the control and treatment groups. The number of fibroblast cells increased in the group treated on the third day and decreased in the group treated on the seventh day.* ***Conclusion:*** *Avocado leaf extract (Persea americana Mill.) accelerates proliferation of fibroblast cells in Wistar rats post-tooth extraction.*

Keywords: *avocado leaf extract, wound healing, fibroblast*

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INTRODUCTION

Within the dental profession, one of the most common procedures performed is tooth extraction which may cause trauma to the blood vessels. After trauma occurs to the blood vessels, the hemostasis process, involving blood clotting on the walls of damaged blood vessels in order to prevent bleeding, commences. The process of post-extraction wound healing can occasionally cause infections, possibly even leading to complications.[1–4] Patients require appropriate post-extraction management in order to reduce the possibility of complications and accelerate blood clotting, thereby promoting wound healing after extraction. The wound healing process itself is relatively complex, consisting of various processes and assisted by many cells, one of them being fibroblasts. Fibroblasts are cells found in connective tissue responsible for the phagocytosis of bacteria. TGF-β (*transforming growth factor β*) and PDGF (*platelet- derived growth factor*) stimulate fibroblast structures to become miofibroblasts located at the edges of ECM which promote wound closure in tissues. Fibroblasts will appear in the wound area after three days with the number of fibroblast cells peaking on the seventh day after trauma.[5–8]

The avocado plant possesses the benefits of traditional remedies[9] since almost all its constituent parts possess properties akin to those of such medicines. The leaves, fruit and seeds all have a high nutrient content. Avocado

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 32a/E/KPT/2017.

DOI: 10.20473/j.djmkg.v51.i3.p140–143

leaves contain a variety of chemicals, including: flavonoid compounds, tannins, katekat, kuinon, saponin, steroids/ triterpenoids, glycosides, cyanogenic compounds, alkaloids and phenols.[7–9]

The aim of this study was to determine the effect of avocado leaf extract on fibroblast proliferation rates and inflamation indicators.

MATERIALS AND METHODS

This study used rodent subjects to evaluate wound healing activity indicated by fibroblast proliferation. Approval by the ethical board was granted (304/ HRECC. FODM/XII/2017). This study used a post-test only control group design with 24 male Wistar rat subjects, 150-200 grams in weight and aged 2-3 months which were allowed to freely consume pellet food for one week.

The sample was divided into four groups, a control group (n=6) and the treatment groups (n=6). In the control group, the subjects were given a 3% CMC Na solution to synchronize the physiological state of their bodies which had no negative effect on their tissues or organs. The treatment groups had avocado leaf extract and 3% CMC Na solution as a 0.1cc solvent inserted into their tooth sockets. Both the control and treatment groups had their mandibula decapitated with all the required specimens being prepared on the 3rd and 7th days of the experiment period.

Fresh avocado leaves were obtained from and identified at UPT Materia Medica, Kota Batu, East Java. The leaves were washed thoroughly, dried and liquified in a blender with 96% ethanol solvent, placed in a tightly sealed jar for 24 hours and agitated in a digital agitator at 50 rpm. The resulting liquid extract was filtered by being passed through a cloth, inserted in an Erlenmeyer tube and subsequently evaporated in a rotary evaporator for 90 minutes and stored in a freezer until required.

A general anesthetic was administered to the subjects by means of chloroform inhalation. Tooth extraction was performed on the left mandibular incisor using pliers after which irrigation was carried out using sterile aquades to remove the remaining debris. In order to stop post-extraction bleeding, a sterile cotton roll was applied to the resulting socket. The treatment protocol adopted was that advocated by Krinke whereby, following removal of the teeth and discontinuation of bleeding from the sockets, the subjects were treated.[10]

The treatment group was selected to have its mandibula decapitated and made into preparations on the 3rd and 7th days. Decapitation of the mandible in the treatment group and preparation on the 3rd and 7th day were performed because fibroblasts appeared in the wound area three days after the trauma before peaking after seven days. On the 3rd and 7th days, a mandibular retrieval procedure was performed by anesthetizing the subjects in a glass gas chamber filled with 10% chloroform. The members of each group had their mandibula decapitated and appropriately disposed of. The decapitated mandibles were made into tissue preparation, before being stained with HE (Haematoxylin Eosin) and observed. Histopathologic observation was performed by counting the number of fibroblasts under a light microscope at 400x magnification. Data was analyzed by means of a One-way Anova test with a 5% significance rate and subsequently with an LSD test to establish whether a significant difference existed.[11,12]

Table 1. Mean amount of fibroblast in the treatment group and control group

Group	X±SD Day 3	X±SD Day 7
K1	3.00^{b}±0.89	14.33^{b}±0.51
K2	20.33^{a}±1.75	10.16^{b}±0.75

Note: Different superscript showed significance difference ($p < 0.001$)

K1: Control group

K2: Treatment group

RESULTS

The results in Table 1 show that after a 3-day experimental period the number of fibroblasts in the treatment group had increased compared to that in the control group (Figures 1 & 2). Conversely, after seven days the number of fibroblast cells in the treatment group was lower than that in the control group (Figures 3 & 4).

Table 1 shows the extent of fibroblast proliferation on day 3 was significantly different in the wounds in the treatment group and the control group, while on day 7 no such significant difference was observed between the two groups.

DISCUSSION

Tooth extraction will result in a wound which then undergoes a healing process consisting of a series of complex processes involving a number of cells, cytokines, growth factors and extracellular components that play a role in repairing damage to the hard tissue and soft tissue.[2] The wound healing process is influenced by several factors including: bacterial infections, damage to the tissue, necrosis, hematoma (tissue bleeding), excessive movement of injured tissue, low blood supply and drug administration.[8,13,14] The injured tissue rapidly experiences an acute inflammatory reaction. The inflammatory phase precedes healing and wound immobilization. The instantaneous acute inflammatory phase is characterized by the exudation of plasma proteins and neutrophils. The chronic inflammatory phase is characterized by the presence of chronic inflammatory cells (macrophages, lymphocytes, and plasma cells).[7] In this study, it was observed that the

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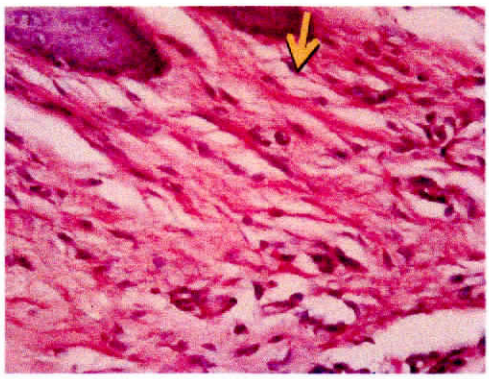

Figure 1. Fibroblasts HPA in control group; 3rd day (400x magnification)

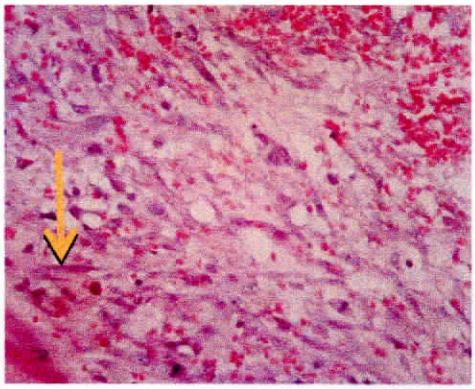

Figure 3. Fibroblasts from the control group; 7th day (400x magnification)

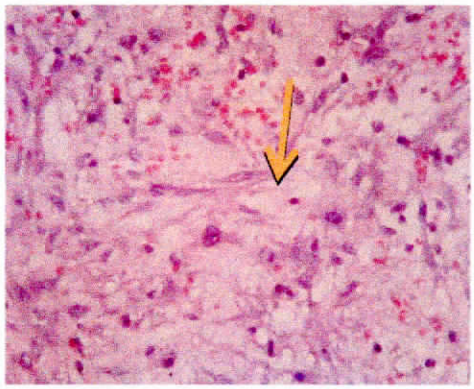

Figure 2. Fibroblasts after the application of avocado leaves extract for 3 days (400x magnification)

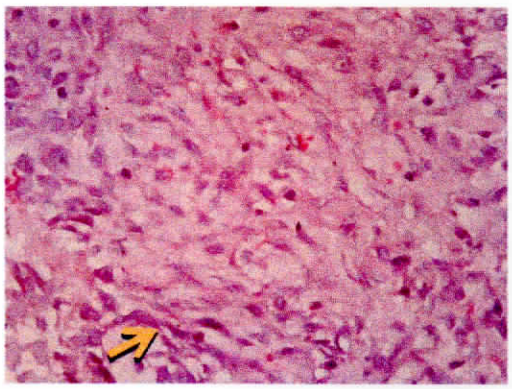

Figure 4. Fibroblasts after the application of avocado leaves extract for 7 days (400x magnification)

proliferation phase begins on the third day and can last for several weeks.[2] In the proliferation phase, neutrophil cells digest bacteria, then release intracellular enzymes into the surrounding matrix before expiring. Monocytes will move from the blood capillaries into the ECM, transforming into macrophages which are then mediated by the inflammatory mediator TGF β. TGF β activates fibroblast cells and stimulates collagen deposition by increasing collagen synthesis. With the synthesis of collagen by fibroblasts, the formation of the epithelial layer will be enhanced by regulating the balance between it and the granulation tissue.[10,15,16] As a result, the mucous epithelium and collagen layer will form.[2]

Acceleration of the wound healing process can be confirmed by the presence of several indicators, one of which is the number of fibroblasts. Fibroblasts are key to the proliferative phase of wound healing, such as destroying fibrin clot, forming collagen, elastin, glycosaminoglycan and proteoglycans induced by TGF-β to form a new extracellular matrix to close the wound and affect the reepithelization process in the wound.[10] Thus, as indicated in this study, the more fibroblasts appear in the socket sample, the more rapid the wound healing process might be.[2]

This study showed that on the third day an increase in the number of fibroblast cells occurred due to active substances such as flavonoids contained in the avocado leaves (*Persea americana* Mill) that have an anti-inflammatory effect through inhibition of cyclooxygenase and lipoxygenase. In this manner, they are able to limit the number of inflammatory cells that migrate to the wound area. Flavonoids play an important role in maintaining permeability and increasing capillary vascular resistance. Therefore, flavonoids are present in pathological conditions such as disruption to the permeability of the blood vessel walls. Flavonoids and phenol substances in avocado leaves accelerate wound healing through antioxidant mechanisms which inhibit the activity of free radicals to donate hydrogen atoms and bond to unstable free radicals that can cause damage to cell membranes and impede cell functioning. The existence of this bond will render free radicals more stable, thereby reducing damage to cell membranes and enabling the proliferation phase to proceed more rapidly. This reduces the duration of the inflammatory reaction, induces earlier TGF-β proliferation and results in the production of fibroblasts. In addition, avocado leaves also contain tannins which are active substances that increase the formation of fibroblast cells and capillary blood vessels,

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causing growth factor to stimulate the proliferation of fibroblast cells.[1,13,17]

Other content of avocado leaves (*Persea americana* Mill) includes saponin, another active substance, which increases monocyte proliferation and can augment the number of macrophages that will secrete growth factors such as EGF, FGF, PDGF and TGF-β. These, in turn, can stimulate the migration to and proliferation of fibroblasts in the wound area in order to more rapidly synthesize collagen.[1,15]

This study showed a decrease in the number of fibroblast cells on the seventh day. Due to a significant increase in fibroblast cell production on day 3, fibroblasts are sufficient to synthesize collagen. This has the result that, on day 7, the number of fibroblast cells decreases as they are transformed into myofibroblasts located on the ECM margins of wound tissue closure.[10,18]

This study showed that avocado leaves (*Persea americana* Mill) topically applied to the post-extraction socket were capable of accelerating the amount of fibroblast present in the wound healing process in Wistar rat tooth sockets.

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