THE POTENTIAL OF COCOA (*Theobroma cacao L.* ) PODS EXTRACT IN PERIODONTAL DRESSING TO RABBIT GINGIVAL WOUND HEALING

Ahmad Faris Adli Izzuddin¹, Anisa Nurkesuma¹
¹Faculty of Dentistry, Universitas Jember
Jalan Kalimantan No. 37, Jember-Jawa Timur
Email : ahmadfaris16@gmail.com

Abstract

Introduction: Anti inflammatory and antibiotic in periodontal dressing are often added but they cause any allergic reaction. Cacao (*Theobroma cacao L.* ) has potential as natural anti inflammatory, antioxidant, and antimicrobial because it contains polyphenolics as flavonoid or condensed or polymerized tannin. The aim of this study was to determine increase wound healing rate indicated by fibroblast cells number and to determine the most effective percentage level of cocoa pods extract. Materials and methods: This study was experimental laboratories that used post test only control group design. The samples were 36 male rabbits had been given gingiva labial injury. The samples were divided into 4 groups based on percentage of cocoa pod extract addition, there were 0%, 5%, 10%, 15%. Each groups were divided into 3 day decapitation subgroups, they were on day 3, 5, and 7. Result: The results showed difference high fibroblast cells number in day 3 but insignificant. Beside it, there were significant difference decrease of fibroblast cells in day 5 and 7 between second treatment group and third treatment group with control group. Discussion: In this case the catechins, tannins, and anthocyanin content of cacao pod extract were able to suppress inflammatory cells number and free radicals produced during inflammatory phase. Conclusion: The conclusion was addition of cocoa pod extract could potentially increase rabbit wound healing rate and most effective percentage extract to affect fibroblast cells was 15%. Suggestion: Need research percentage addition of extract of the cocoa pod which is different to know lethal dose.

Keywords : Cocoa pods, fibroblast, wound healing, polyphenolics
A. INTRODUCTION

Background

Periodontal dressings is material used for dress the wound after surgical periodontal. The addition of anti inflammatory and antibiotic on periodontal dressings often done but also pose an allergic reaction so we need a alternative material to substitute which it can speed wound healing process up without generate side effects. Plants that could potentially anti inflammatory, antioxidants, and a natural antimicrobial is cocoa (Theobroma cacao l.) because contain polyphenols in form flavonoids or condensed tannins.

Baharudin (1996), granting extract of cocoa pod at concentrations 5%, 10%, and 15% have anti inflammation activity against number of macrophages cells\textsuperscript{[1]}. A active macrophage produce factors chemotaxis, growth factor, and cytokines that affect the proliferation, and migration of fibroblasts, endothelial cells, and epithelial\textsuperscript{[2]}. This indicates that extracts of cocoa pod capable of accelerating the process of wound healing.

Formulation of the Problems

1. Whether extract of cocoa pod addition in periodontal dressing potential for increase wound healing of rabbit gingiva that is viewed from fibroblast cells number?
2. How percentage of pod cocoa extract in periodontal dressings that is effective for increase fibroblasts cells number to wound healing of rabbit gingiva?

Research Purposes

1. To examine the potential addition of cocoa pod extract in periodontal dressing to increase wound healing of rabbit gingiva that is viewed from fibroblast cells number.
2. To know percentage of cocoa pod extract in periodontal dressing that is effective for increase fibroblas cells number to wound healing of rabbit gingiva.

**Benefits Of Research**

This research is expected to provide benefits such as:

1. As an additional information about the impact of cocoa pod extract addition ( *Theobroma cacao* L.) in periodontal dressing to increase speed of wound healing.

2. As a research reference on a dose of cocoa pod extract used ( *Theobroma cacao* L.) to be additional ingredient in periodontal dressing.

**Hypothesis**

The addition of cocoa pod extract ( *Theobroma cacao* L.) in periodontal dressing able to increase the speed of rabbit gingiva wound healing that is viewed from fibroblast cells number.

**B. LITERATURE REVIEW**

**Cocoa (Theobroma cacao L.)**

According to Wollgast and Anklam (2000) in Porbowaseso (2005), classifies of cocoa polyphenols in three groups, catechins (flavan-3-ols) 37%, 4% and proantosianidin relationships 58% \[3\].

Catechin called catechoat acid with the chemical formula $C_{15}H_{14}O_{6}$, is in a State of pure highly soluble in hot water, alcohol, and ethyl acetate\[4\]. The relationships with chemically derived pigment, formed from sianidin sianidin with the addition or reduction of hydroxyl groups or by methylation or a glycosylation. Proantosianidin is another name of
condensed tannins. The tannins are bound with sugars soluble in the solvent condensed tannins, while hidroalkohol or tannins are more easily extracted with the solvent acetone 70%.

**Wound healing**

Wound healing is a dynamic process that includes blood vessels, fibroblasts, epithelial and \[^5\]. The process then happens in the healing of wounds is divided into three phases: the inflammatory phase, a phase of the proliferation, and completion phase \[^6\].

Inflammatory phase lasts for 0-3 days \[^5\]. At the beginning of this phase of the injured area is dominated by platelets. Platelets release a number of factors chemotaxis, growth factors, and cytokines that attract other platelets, and leukocytes to the site of the wound fibroblasts \[^7\]. Next in 24-48 hours phase inflammatory taken over by leukocytes especially pmn, a macrophage, and a lymphocyte played the role to deprive of debris and memfagosit bacteria. Factor chemotaxis, growth factor, and also by a macrophage cytokine that is produced. Growth factor such as PDGF, FGF, EGF, TGF α dan TGF β impact on migration and the proliferation of fibroblas, endothelial cells, and epithelial \[^2\]. Next phase proliferation between 3-24 day depends on the size wound. This phase dominated by tissue formation granulation, synthesis collagen by fibroblas, and process epitelisasi \[^8\]. Last phase remodeling tissue, consists of three part, namely epitelisasi contraction, and reorganization of connective tissue. Duration phase it began at the 3rd sunday and lasting minimum 1 year \[^9\].

**Fibroblast cells**

Fibroblasts are the cells that produce fibers and ground substance of the connective tissue amorphous. Fibroblasts was instrumental in the formation of extracellular matrix
components of connective tissue such as the synthesis of collagen, elastin, glikosaminoglikan, proteoglycans, and glycoproteins multiadhesif[10].

**Periodontal Dressing**

Periodontal dressings is material for dress the wounds after surgical periodontal. Periodontal dressing does not contain material that could in healing, but only help healing for the hurt protected[11].

Periodontal dressing does not contain of eugenol often used because does not have an irritant effect. A formula was introduced by Baer based on the reaction between a metallic oxide with fatty acids[12].

**C. RESEARCH METHODS**

The research was experimental laboratories with the post test only control group design. Population samples and object were 36 of local guinea (oryctolagus cuniculus). The samples divided into four groups based on the percentage of an cocoa extract in periodontal dressing that were control group (0 %), treatment group one(5 %), group two (10 %) and group three(15 %) with each group consists of 9 rabbits. The treatment of each group divided into subgroups of dekapitasi, day namely subgroup one (3rd), subgroups two (5th day), and subgroups three (7th day) with each group consists of 3 rabbits.

The stage of making cocoa cocoa pod extract was blend the cocoa skin to get a fine powder. A fine powder of cocoa pod was remaseration with solvent acetone 70 % as much as three times. Filtrat obtained then the solvent was evaporated with rotavapor until not left
and obtained extract liquid. Extract liquid concentrated by an oven at a temperature of 60°C [13].

Stage making periodontal dressings extract skins of cocoa:

a. The control group (K): periodontal dressings formula Baer already homogeny 100 grams without extra extract rind of cocoa.

b. Treatment group one (KP1): formula periodontal dressing formula Baer already homogeny 95 grams and added extract rinds of cocoa stored 5 grams.

c. Treatment group two (KP2): periodontal dressing formula Baer already homogeny 90 grams and rind cocoa added extract as much as 10 grams.

d. Treatment group three (KP3): periodontal dressing formula Baer already homogeny 85 grams and added extract rinds of cocoa 15 grams

Treatment of animals exercised after adapted for 7 days, then performed anesthesia in combination with ketamin and xylazine, punch biopsy and conducted on the gingiva of dental insisivus right lower jaw section with diameter 2.0 mm labial until it reaches alveolar bone. The wound was closed with periodontal dressings with cocoa skin extract content. Each group is divided into three sub groups according to the day of dekapitasi, on day 3, day 5, and the 7th day. Then do the making of preparations with Mallory and repainting Trichrome fibroblasts cells observed with the light microscope with 1000x magnification.
D. RESULT AND DISCUSSION

Result

Based on the research that has been done, the average fibroblasts number cells of rabbits in the control group (K), treatment group one (KP1), two (KP2), and three (KP3) can be seen in attachment 1. Comparison of the average fibroblasts number cells of each group can be seen in attachment 2.

The Data obtained was tested with Kolmogorof-smirnov test, shows a normal data distribution with the value significance of 0.308 (P>0.05). Further Data was tested with test Levene, shows data is not homogeneous with the significance value of 0.018 (P<0.05). Then The Data was tested with a non-parametric test of Kruskal-Wallis, the result was a difference with a significance value of 0.001 (P<0.05). The next test was a statistical test of the Mann Whitney to find out which groups have significant differences. Mann Whitney test results can be seen in attachment 3.

Discussion

On the day 3 of the treatment group showed a proliferation of fibroblasts cells amounts was higher even though less significant compared to the control group. This happens because the migration and proliferation of fibroblasts cells have started to take place at the inflammatory phase that is on a initial 24 hours post treatment. Migration and proliferation of fibroblasts cells induced by growth factors PDGF and TGF- TGF-β and cytokine factors released by platelets and leukocytes\textsuperscript{[5]} \textsuperscript{[7]} \textsuperscript{[9]}. In this case the catechins, tannins, and anthocyanin content of the cacao pod extract were able to suppress
inflammatory cells number and free radicals produced during inflammatory phase. On the dose addition of as much as 5%, 10%, 15%, and catechins, tannins, and antiosisnin gives a bitter taste but the effects did not inhibit the inflammatory process continuity that occurs naturally. It can be concluded that indirectly, catechins, tannins, and the relationships were able to increase the activity of fibroblasts proliferation and migration.

On the day 5 and 7, the average number of fibroblasts cells decreased. There was a significant difference in treatment group two (KP2) and treatment group three (KP3) compared to the control group (K), this happens because the activities of fibroblasts cells are more progressive in synthesis of collagen fibers and the occurrence of cells differentiation of fibroblasts into miofibroblas so that in one point of view of microscope would appear fewer of fibroblasts number cells that can be observed in attachment 5. That was caused by catechins, tannins and anthocyanin contained in the extract of the cocoa pod in periodontal dressing. The presence of condenced tannins, catechins, and influencer relationships with a bitter taste, antibacterial, antioxidant and [14].

As an anti-inflammatory agent, tannin works by inhibiting arakhidonat acid [15]. Anthocyanin and catechin also acted as anti inflammatory agents which in high concentrations block line siklooksigenase and phospholipase A2, while in low concentrations of this compound, just block sikloogsigenase path. Inhibition of primarily from arachidonic acid release from inflammatory cells will lead to less availability of substrate arachidonat for siklooksigenase and lipooksigenase lines, which will ultimately suppress the amount of prostaglandins, prostacyclins, thromboxanes and endoperoksida on one side and acid hydroperoxide, hidroksieikostrenoat acid, and leukotrienes on the other [16]. Less availability of substrate arakhidonat for the siklooksigenase and lipooksigenase
process resulting in decreased inflammation characterized by a decrease of inflammatory cells number microscopically on lesions area \(^{[17]}\). Reduction of inflammatory cells cause cytokine produced also reduced so that free radicals released by cytokines also decreases. The decline in number of these free radicals and collagen fibroblasts synthesis activities may soon take place.

As antibacterial agents, tannin works by coagulation or agglomerate bacteria protoplast, thus formed a stable bond with the bacterial proteins \(^{[18]}\). In addition, catechin have a tendency to bind bacterial proteins, thus interfering metabolism of bacteria. Potential of cocoa pods extracts as antibacterials cause inflammatory cells to phagocytes bacteria become easier, so that free radicals released by cytokines are not excessive. Therefore inflammatory phase is short, so it can proceed with proliferation or fibroplasia phase.

As an antioxidants agent, catechin is able to act as antioxidants for inflammatory phase. Free radicals are produced during the inflammatory, a free-radical types of reactive oxygen species (ROS). The working mechanism of catechins in neutralize ROS is through –OH group, so the radicals become pepsinogen. That process is \(\text{Catechin} (-\text{OH}) + R (\text{Free radical}) \rightarrow \text{Catechin} (\text{O}^{2+}) + RH\)^{[19]}\). The neutralizing cause lowering expression of MMP-1, MMP-8, MMP-9, and so barriers on degradation of type-3 collagen and matrix extracellsular is synthesize by fibroblasts caused by ROS directly or by activated MMP.

The effect of antioxidant, antibacterial, and anti inflammatory work together during inflammatory process. They worked together in lowering effect of excessive inflammation so inflammatory phase can last a short time. The next healing phase continues with the
proliferation phase is marked by the increasing activity of fibroblasts in the synthesis of collagen fibers.

E. CONCLUSION AND ADVICE

Conclusion

The addition of extract pod of cocoa (*Theobroma cacao* L.) in periodontal dressing can potentially increase the speed of wound healing with a percentage that most effective way to increase fibroblasts cells number in rabbit gingiva injuries is 15%.

Suggestion

Advice that can be given of this research.

1. Need to reserch the cocoa pod active substances that play a role in wound healing.
2. Need to research the percentage addition of extract of the cocoa pod which is different to know the lethal dose of this material.
REFERENCE

ATTACHMENT

ATTACHMENT 1. THE AVERAGE NUMBER OF RABBITS FIBROBLAST CELLS

Table of average number of rabbits fibroblasts cells in the control group (K), treatment group one (KP1), two (KP2), and three (KP3) after administering treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Amount fibroblast day- ((X\pm SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>K</td>
<td>8,222±1,71053</td>
</tr>
<tr>
<td>KP1</td>
<td>8,888±0,38490</td>
</tr>
<tr>
<td>KP2</td>
<td>9,000±0,33333</td>
</tr>
<tr>
<td>KP3</td>
<td>9,111±0,83887</td>
</tr>
</tbody>
</table>

\(X\pm SD\) : average fibroblast cells number±deviation standard

ATTACHMENT 2. IMAGE GRAPHS OF AVERAGE FIBROBLAST CELLS NUMBER

Image graphs of average fibroblasts cells number in the control group (K), treatment group one (KP1), two (KP2), and three (KP3) after administering treatment
ATTACHMENT 3. RESULT OF MANN WHITNEY TEST

The table result of Mann Whitney test that rabbit macrophage cells number on treatment group one (KP1), two (KP2), three (KP3), and four (the garden) after administering treatment (attachment 4).

<table>
<thead>
<tr>
<th></th>
<th>KH3</th>
<th>KP1H3</th>
<th>KP2H3</th>
<th>KP3H3</th>
<th>KH5</th>
<th>KP1H5</th>
<th>KP2H5</th>
<th>KP3H5</th>
<th>KH7</th>
<th>KP1H7</th>
<th>KP2H7</th>
<th>KP3H7</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH3</td>
<td>-</td>
<td>0.817</td>
<td>0.658</td>
<td>0.513</td>
<td>0.072</td>
<td>0.077</td>
<td>0.050</td>
<td>0.050</td>
<td>0.046*</td>
<td>0.046*</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>KP1H3</td>
<td>-</td>
<td>0.637</td>
<td>0.825</td>
<td>0.043*</td>
<td>0.046*</td>
<td>0.046*</td>
<td>0.043*</td>
<td>0.046*</td>
<td>0.043*</td>
<td>0.046*</td>
<td>0.046*</td>
<td>0.046*</td>
</tr>
<tr>
<td>KP2H3</td>
<td>-</td>
<td>1</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
<td>0.046*</td>
<td>0.046*</td>
<td>0.046*</td>
<td>0.050</td>
<td>0.050</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KP3H3</td>
<td>-</td>
<td>0.046*</td>
<td>0.050</td>
<td>0.050</td>
<td>0.046*</td>
<td>0.046*</td>
<td>0.050</td>
<td>0.050</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KH5</td>
<td>-</td>
<td>0.369</td>
<td>0.046*</td>
<td>0.046*</td>
<td>0.046*</td>
<td>0.099</td>
<td>0.043*</td>
<td>0.046*</td>
<td>0.046*</td>
<td>0.046*</td>
<td>0.046*</td>
<td></td>
</tr>
<tr>
<td>KP1H5</td>
<td>-</td>
<td>0.050</td>
<td>0.050</td>
<td>0.268</td>
<td>0.046*</td>
<td>0.050</td>
<td>0.050</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KP2H5</td>
<td>-</td>
<td>0.822</td>
<td>0.825</td>
<td>0.369</td>
<td>0.184</td>
<td>0.184</td>
<td>0.077</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>KP3H5</td>
<td>-</td>
<td>0.487</td>
<td>0.637</td>
<td>0.184</td>
<td>0.077</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KH7</td>
<td>-</td>
<td>0.197</td>
<td>0.046*</td>
<td>0.046*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KP1H7</td>
<td>-</td>
<td>0.178</td>
<td>0.072</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>KP2H7</td>
<td>-</td>
<td>0.261</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>KP3H7</td>
<td>-</td>
<td></td>
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<td></td>
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<td></td>
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</tr>
</tbody>
</table>

H3 declared to day 3 observation, H5 declared to day 5 observation, H7 declared to day 7 observation. Sign (*) indicates the data in the statistical continuation has values of significance (P<0.05;).

ATTACHMENT 4. MAKING OF PERIODONTAL DRESSING

Manufacture of rosin: pour powder as much as 28.5 grams and zinc oxide as much as 21.5 grams. Mix the rosin and zinc oxide until homogeneous. The manufacture of pasta: pour fat and hydrogenated 47.5 g 2.5 g zinc oxide then mix until homogenous. The making of periodontal dressing: mix powder 50 grams and 50 grams of pasta little by little until homogeneous (100 grams).
ATTACHMNET 5. IMAGE OF RESULT

An arrow indicates fibroblasts cells magnification trichrome mallory 1000x
Preparat Day 3 (A: control group; B: Treatment control group 1; C: Group Treatment 2; D: 3 Treatment Groups)

An arrow indicates fibroblasts cells magnification trichrome mallory 1000x
Preparat Day 5 (A: control group; B: Treatment control group 1; C: Group Treatment 2; D: 3 Treatment Groups)
An arrow indicates fibroblastic cells magnification trichrome mallory 1000x
Preparat Day 7 (A: control group; B: Treatment control group 1; C: Group Treatment 2; D: 3 Treatment Groups)