

The Effect of *Carica Pubescens* Lenne and K. Koch Fruit Extract from Dieng Plateau and Cangar to the Amount of Fibroblasts Cells on the Healing of Oral Mucosal Inflammation

Risma Aprinda Kristanti

Department of Biology, Faculty of Science and Technology, Maulana Malik Ibrahim State Islamic University of Malang, Indonesia

*Email : risma.aprinda@yahoo.com

ABSTRACT

The purposes of this research are to know the effect of *C.pubescens* fruit extract on the amount of gingival fibroblasts in wound healing of *Rattus norvegicus* mouth cavity's mucosal and to know the effect of *C.pubescens* comes from two different areas (Dieng and Cangar) on the amount of gingival fibroblasts in wound healing of *Rattus norvegicus* oral mucosa. Twenty eight rats are divided to be four groups (K1, K2, K3, and K4), each rat is wounded 1 cm on the gingival mucosa of lower jaw (specifically on the apical region of incisive teeth). K1 is the control group with aquadest treatment on the wound. The wound in the K2 is treated with *C.pubescens* fruit extract from Dieng. The wound in the K3 is treated with *C.pubescens* fruit extract from Cangar. And the treatment for K4 is medicated by policresulen (common medicine for oral mucosal wound). On the fifth day of the treatment, all rats are sacrificed, and the gingival tissue is taken up for the next step. Gingival tissue is smeared by Haematoxylin Eosin (HE) to analyze the amount of gingival fibroblasts histologically. The result of this research shows that the highest average amount of gingival fibroblasts comes from K4 (policresulen treatment). And there is no significant difference on the number of *Rattus norvegicus* gingival fibroblasts from all of the groups (K1, K2, K3, and K4).

Keywords: *fibroblast, gingival, wound, mucosa, mouth, C.pubescens*

INTRODUCTION

The oral mucosa wound is one kind of lesion that commonly known on mouth cavity. The wound of oral mucosal is often neglected without medication, and it disturbs the activity of the oral cavity (e.g. mastication and speaking). There are so many cause of traumatic lesion, including dentistry procedures that are like chemical trauma caused by root canal irrigation and local anaesthetic procedures for lidocaine with HCl 2% (Lacy, 2009).

In each wound healing process is found three main components, which are: (1) tissue basic material that contains acid mucopolysaccharide, (2) new capillaries that are produced by endothelial proliferation of injured capillaries, and (3) fibroblast that produces collagen fibers (Boyne, 1966). If there are so many fibroblasts formed, it will cause fibroblasts accumulation on slit wound, and then so many collagens are formed too (Ozgenet *et al.*, 2001). For 48 hours after the wounding, monocyte is activated, and then transformed into macrophage. After that, macrophage releases growth factor that can attract fibroblasts and smooth muscle cells into the area of injury. In this proliferation phase, fibroblast has main function on the wound healing process (Diegelmann, 2004).

Indonesian nation made up of various ethnic groups, it has biodiversity of traditional

medicines that made from Indonesian natural ingredients with the number of biodiversity more than 30.000 species of plant and 940 species are known as herbal medicine (Maheswari, 2002).

Allah says in Surah Al An'am verse 99:

وهو الذي أنزل من السماء ماء فأخرجنا به نبات كل شيء فأخرجنا منه خضرا نخرج منه حبا متراكبا ومن النخل من طلعها قنوان دانية وجنات من أعناب والزيتون والرمان مشتبها وغير متشابه انظروا إلى ثمره إذا أثمر وينعه إن في ذلكم لآيات لقوم يؤمنون

The meaning: "And He who sends down rain from the sky, and We grow many plants, green plants. We sends out grains from the green plants, and stalks are dangle from the flower dates, there are gardens of grapevines and olives and pomegranates, similar yet varied. Look at (each of) its fruit when it yields and (at) its ripening. Indeed in that are signs for a people who believe." (QS. Al An'am:99).

The wound healing process is influenced by some compounds that could be find in the herbal medicines, including saponin, flavonoid, essential oil, protein, and vitamin C (Sudarsono *et al.*, 2002). Maheswari (2002) defines herbal medicine is medicine come from nature, without artificial compound, and can be common medicine that is used traditionally, but it could be made by modern method.

The morphology character, antioxidant capacity, and protein banding pattern analysis on *C. Pubescens* have been studied (Laily, 2011), but specific research about the active compound of *C. Pubescens* to be drug raw material and its conservation have not been studied yet. The fruit of its plant contains flavonoid and saponin. Flavonoid is closely associated with antioxidant activity (Minarno, 2014). And saponin can support the wound healing process because of its ability to prevent inflammation and as antibacterial agent. The infection may extend the inflammation time. Saponin modifies the expression of TGF- β receptor, so the fibroblast may accelerate stimulation of fibronectin synthesis (Kanzaki *et al.*, 1998). The wound healing process is influenced by migration and proliferation of fibroblasts in the area of injury.

The difference height of planting area may determine the factor of environmental climate and metabolism process in the plant. The weather is the condition of atmosphere at a certain time that can change from time to time, whereas climate is the average of weather condition in the long term (at least 30 years constantly) (Kartosapoetra, 2004).

The climate variability among regions are controlled by some natural factors, one of them is the altitude (the height of planting area from sea level) that effect on temperature. Braak formula ($t_0 = (26,3 - 0,61 h)^\circ\text{C}$) says that higher place measured from sea level has lower temperature (Steenis, 1972). The effect of climate on plant is started by the direct influence of weather, including radiation and temperature on photosynthesis, and another metabolism process in the cells of plant organ. Photosynthesis and respiration are the beginning of the process of the life, these two process are taking simultaneously, but the photosynthesis takes place with the aid of the light (can be sunlight or lamps), whereas the respiration takes place continuously (Fitriningrum *et al.*, 2013).

Based on the description above, researcher assumes that the wound healing on oral mucosa is accelerated by the treatment topically with the *C. Pubescens* fruit extract. This is caused by saponin content in the *C. Pubescens* fruit that can support the process of fibroblast formation as one step of the wound healing process in oral mucosa. In addition, the difference planting

area of *C. Pubescens* will influence its compound, and it will give different therapy effect on wound healing process.

MATERIALS AND METHODS

The kind of this research is laboratory experimental research, the research design is “*the post tests only control group design*”. This research conducted in Animal Physiology laboratory and Optical Laboratory of UIN Maulana Malik Ibrahim Malang, and also Histology Laboratory of Brawijaya University, Malang.

Sample

Sample used in this research are 28 health white rat (*Rattus norvegicus*) aged 40-60 days with the weight 150-200 grams. The rats have not been used for research.

The sample is divided in to 4 groups; they are K1, K2, K3 and K4. The K1 is control group with oral mucosal wound treated by aquadest topically. In the K2, the rats with oral mucosal wound are treated by *C.pubescens* fruit extract (from Dieng) topically. The rats in the K3 group are treated with *C.pubescens* fruit extract (from Cangar) topically on the oral mucosa wound. The last one is K4, the rats on this group are treated with policresulen preparation (common medicine for oral mucosal wound) topically on the oral mucosa wound.

Induction of the Oral Mucosa Wound

Induction of the oral mucosa wound is created by incision with mess number 3 on the labial mucosal of the rat (under the incisive teeth on the lower jaw). One centimetre incision is made to reach the alveolar bone. After the incision made, peroxide solution (H₂O₂) 30% is applied in the injured area for 3 second by *cotton pellet* to accelerate wound forming.

The Treatment with C. pubescens Fruit Extract

The concentration of *C. pubescens* fruit extract used in this experiment is 100%. *C. pubescens* fruit extract is made by maceration method with ethanol as the solvent. The treatment with *C. pubescens* fruit extract is treated one day after the wound made till 5th day by micropipette in the wound area. One hundred (100) µl of *C. pubescens* fruit extract is given in the K2 and K3 at each rat. In the K2 is treated by *C. pubescens* fruit extract from Dieng, whereas in the K3 is given *C. pubescens* fruit extract from Cangar.

Data Retrieval

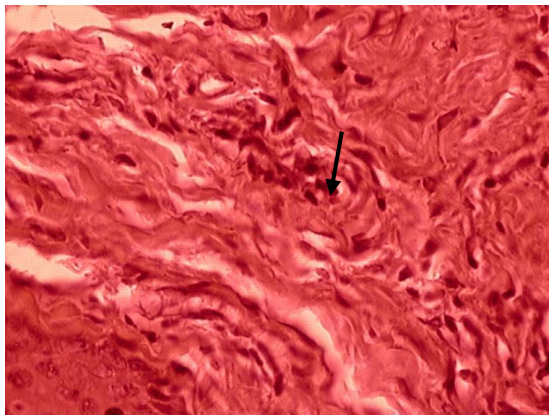
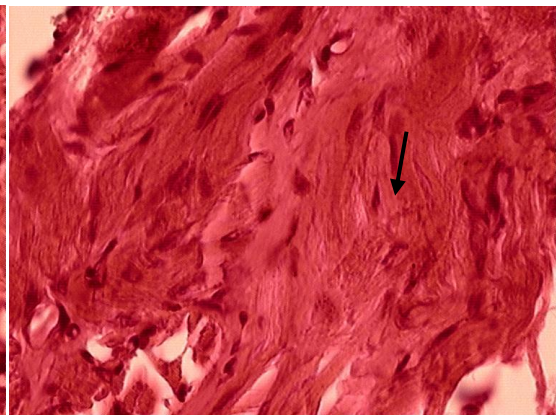
In the 6th day, all the rats are sacrificed and the labial gingival mucosa of the lower jaw are taken for histological preparations. After the histological preparation created, the fibroblast cells are counted. The data obtained are tested the normality by *Kolmogorov-Smirnov*. ANOVA test is done to look at the differences in the number of fibroblasts between groups.

RESULT AND DISCUSSION

Based on the research conducted, the number of fibroblast in each group is represented in the table 1.

Table 1. Average value (*mean*) and standard deviation (SD) of the number of fibroblast in the K1, K2, K3, and K4

<i>Group</i>	<i>N</i>	<i>Mean and SD</i>
K1	7	93,00 ± 109,423
K2	7	148,00 ± 73,903
K3	7	164,43 ± 135,520
K4	7	225,29 ± 100,589
Total	28	157,68 ± 111,847

**Fig. 1.****Fig. 2.**

Note: the arrow at the figure 1 and figure 2 shows the fibroblast formed in the gingival wound healing of the rat.

First, the data is tested the normality by *Kolmogorov-Simonov* and it shows $p > 0,05$; so it can be concluded that the data is distributed normally, and then it is tested the homogeneity by *Levene test*. The result of this test shows $p > 0,05$, it means that the data is homogeneous. After that, to know the difference the amount of the fibroblast cells between experimental groups is

analyzed by ANOVA test. From the ANOVA Test results showed that the number of fibroblasts is no significantly different between the groups with $p > 0,05$.

To know the groups who have difference amount of the fibroblast cells with normal distribution and the homogeneous variation, the groups are tested by LSD test. The result of the test is represented on table 2 below:

Table 2. The LSD test result with difference the number of fibroblast in the K1, K2, K3 and K4

<i>Group</i>	<i>P</i>	<i>Information</i>
K1 and K2	0,346	Not significant
K1 and K3	0,224	Not significant
K1 and K4	0,030	Significant
K2 and K3	0,777	Not significant
K2 and K4	0,190	Not significant
K3 and K4	0,299	Not significant

The table above shows that there is no significant difference for the number of gingiva fibroblast between groups, except for K1 and K4.

Discussion

Carica pubescens Lenne & K. Koch is one kind of plateau plant in Indonesia with high concentration of Vitamin C that potentially to be herbal medicine in the wound healing of oral mucosal. In Indonesia, *C. Pubescens* can be found in the plateau, for example in the Dieng Plateau (Central Java) and in the Cangar Plateau (Batu, East Java). *Carica pubescens* Lenne

& *K. Koch* is a species that can be adapted in the place with an altitude of 1.400-2.400 metres above sea level, low temperature, and high rainfall.

Some components of *Carica pubescens* has significant activity in the wound healing, including essential oil, poliphenol, flavonoid, and saponin. Essential oil acts as an antibacterial, so it can accelerate neutralization of foreign material (Parwata and Dewi, 2008). Poliphenol acts as an antihistamine. While flavonoids are useful to for protecting the cell structure, synergistic activity with vitamin C (increase the effectiveness of Vitamin C), prevent bone loss, antibiotic, and as anti-inflammatory agent. In addition, *Carica pubescens* also contain Vitamin A (Ahkam, 2008; Hidayat, 2001). Saponin in the plant can support wound healing by preventing infection, because saponin has anti-inflammatory activity and antibacterial agent. The infection will extend the inflammatory phase. Saponin modifies the expression of TGF- β receptor, so the fibroblast can accelerate the stimulation of fibronectin synthesis (Kanzaki *et al.*, 1998). There are some compounds in *C. pubescens* possible to be herbal medicine that can increase the number of gingival fibroblast in the wound healing. This research aims to examine more deeply about the effect of *C. pubescens* fruit extract from Dieng plateau and Cangar on the amount of gingival fibroblast cells in the wound healing of oral mucosa at male rats strain Wistar (*Rattus norvegicus*).

This research is a kind of laboratory experimental research that qualifies as true experiment. The treatment of the research sample is conducted in provided room and the examination of measurable variables are conducted in the laboratory (Zainuddin, 2000).

This research design used is *post test only control group design*, because of the experimental animals are divided to be 4 groups by random allocation technique and the data of variable depends on each sample that is obtained after the treatment (Zainuddin, 2000).

The result of descriptive analysis shows that the average number of gingiva fibroblast in the control group (K1) is 93. Whereas K2, K3, and K4 are 148; 164,43; 225,29 respectively. It indicates that the number of gingival fibroblast in K4 (treatment with policresulen) is higher than in others group. Control group has lower number of fibroblast. It is caused by selectivity effects of policresulen only in the injured tissue, i.e. coagulation, and then issued or released. Whereas healthy squamous epithelium not affected by this drug. In direct contact, policresulen can be deadly pathogenic flora, but instead maintain normal flora. Even this medicine efficacious as strong astringent and strong haemostatic activity, so it can be accelerate the formation of fibroblast.

The number of gingival fibroblast in K2 (treatment with *C. pubescens* fruit extract from Dieng) and K3 (treatment with *C. pubescens* fruit extract from Cangar) groups are higher than in the control group. Enoch and Harding (2003) say that fibroblast proliferation can be stimulated by IL-1b naturally. Kanzaki *et al* (1998) confess that saponin content in *C. pubescens* can increase the density of fibroblast with the synthesis, secretion, and TGF- β activation. Platelet aggregation process secretes TGF- β which can be stimulated by *fixed oil* (Andajani and Maharddika, 2003), as well as possible to achieve optimal levels at a concentration of 40%. Mimica-Dukic (2003) confess that enhancement of *oil* content in the *C. pubescens* fruit extract in accordance with a reduction ratio between powder and water. TGF- β stimulation accelerates the fibronectin activity in the formation of fibrin clots (Mitchel and Cotran, 1997). In the early stages of wound healing, saponin may increase the fibronectin synthesis (Kanzaki *et al.*, 1998). Fibrin clots formed by the increasing activity of fibronectin will be the

framework for re-epithelialisation and fibroblast proliferation. Thus when a fibrin clots are formed rapidly, then fibroblast will soon proliferate to the wound area to hold tissue recovery.

From the descriptive analysis record shows that the number of rat gingival fibroblast in the group which have been treated by *C. pubescens* fruit extract (from Cangar) is higher than the number of fibroblast in the group with *C. pubescens* fruit extract (from Dieng) treatment. It is caused by the difference of *C. pubescens* fruit maturity whether from Cangar or Dieng. According Abidin (1991), the changes occur during fruit ripening process, including changes in the structure, texture, colour, taste, and its biochemical reaction. It also influence the compounds contained in *C. pubescens* fruit.

Besides that, in a process of plant metabolism, temperature is one kind of factor that influence the respiration rate (tends to decrease at low temperature), so the photosynthesis product is converted into another form. Plant produces antioxidant (i.e. Vitamin C) and also develops an environment with high acid content to adapt to the extreme environment. In the carbohydrate metabolism is explained that Vitamin C is synthesized from monosaccharide by enzymatic biochemical reaction that convert D-Glucosa-6-P to be vitamin C (L-Ascorbic acid) (Wheeler *et al.*, 1998).

Whereas ANOVA test result shows that there is no significant difference in the number of gingival fibroblast among the groups, except between control group and group with policresulen treatment. It is caused by no longer therapy time, so the number of fibroblast formed has not been able to indicate a significant difference among the groups. Fibroblast will become dominant cell at 7-14th day after injury (Torre, 2006). But, in this research, the gingival tissue is taken at 5th day to histological examination.

Advice that can be given is needed further research about the effect of altitude planting and the climate of the planting on the active compounds of *C. Pubescens* which useful for wound healing process in the oral mucosa. Besides that, further research is needed to determine optimal dose of *C. Pubescens* fruit extract that can be accelerate the process of fibroblast formation in wound healing of oral mucosal.

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