

# TOPICALLY ADMINISTRATIONS OF 50% FENNEL'S ETANOL EXTRACT ENHANCE NEOANGIOGENESIS AND REEPHITELIZATION IN HEALING TRAUMATIC ULCER ON ORAL MUCOSA MALE RAT

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## ABSTRACT

Dental procedure sometimes can cause injury to the oral mucosa intentionally or unintentionally. Traumatic ulcer are open sores that are often found in the oral cavity. Clinical features such as single mucosal ulceration that can be caused by physical or mechanical trauma, temperature changes, chemicals and radiation. The purpose of this study is to prove whether the 50% of ethanol fennel extract (*Foenicullum vulgare* Mill.) more effective in improving angiogenesis and reepitelialization than Povidone Iodine for healing traumatic ulcer on oral mucosa white male rats.

This study using the approach method Randomized Post Test Only Control Group Design on 32 male rats, divided into two treatment groups, each group of 16 rats. Control group get Povidone Iodine administration for 3 days, and the treatment group was given 50% of fennel ethanol extract for 3 days. On 7th days rats euthanized to collect the oral mucosa tissue and then made histology preparations with HE staining.

The data obtained were analyzed, using normality test by Shapiro-Wilk and followed by independent T-test. Results The mean neoangiogenesis and reepitelialisasi significantly different ( $p < 0.05$ ).

It was concluded that the 50% ethanol extract of fennel which contains flavonoids, saponins, tannins and vitamin C can increase the neoangiogenesis and reepitelialization than Povidone Iodine on traumatic ulcer healing in oral mucosa white male rats.

Keywords: ethanol extract of fennel's (*Foenicullum vulgare* Mill.), Povidone Iodine, angiogenesis, reepithelialization, traumatic ulcer

## INTRODUCTION

Dental procedure can sometimes cause injury to the oral mucosa intentionally or unintentionally. Traumatic ulcer on oral mucosa are open sores that are often found in the oral cavity. The presence of traumatic ulcer sometimes very disturbing during the process of

chewing, talking, and even interfere with activities of cleaning the oral cavity, causing discomfort, pain and burning sensation in patients with traumatic ulcer.

traumatic ulcer on oral mucosa is a oral lesion that common occurs in most people at different ages and genders. Ulcer is a epithelial damage usually ovoid and has yellowish-white

necrotic center surrounded by a broad erythematous border. Traumatic ulcer can be symmetrical or asymmetrical, its size depending on the caused trauma, and usually pain. These lesions are caused by mechanical trauma and there are relation between trauma and lesion. Traumatikus ulcer frequently found on the buccal mucosa and labial<sup>123</sup>.

Research conducted by Castellanos 2003 in Mexico showed the prevalence of ulcers traumatikus is 40.24% in his study in Turkey found the prevalence of traumatic ulcers is 30.47%<sup>45</sup>. Traumatic ulcer can occur due to mechanical stimuli, such as contact with sharp food, trauma when brushing teeth, biting while eating or talking. Traumatic ulcer is a single ulceration of the oral mucosa which can be caused by physical or mechanical trauma, temperature changes, chemicals and radiation<sup>1</sup>. Treatment of patients with traumatic ulcers are symptomatic aimed to reduce inflammation, pain and improve healing proces of the lesion area<sup>6</sup>.

Wound healing is a complex process involving many physiological process. immunological cell fight infection and dispose of damaged tissue. Blood supply was re-established in the area of healing through angiogenesis. cell proliferation and fibroplasia replace tissue damaged. Wound closure occur by the migration of epithelial cells. A safe treatment should encourage the healing process or at least not inhibit this process<sup>7</sup>.

Povidone Iodine solution is a relatively safe treatment for minor acute wounds. Povidone Iodine is used in the

treatment of wounds but can cause dermatitis contact on the skin, have toksikogenik effects on fibroblasts and leukocyte, inhibits migration of neutrophils and monocytes decrease<sup>8</sup>.

A better alternative treatment for wound healing may be available, many substances such as tissue extracts, vitamins and minerals, plant products have been reported to have a healing effect<sup>9</sup>.

The development of medical science in Indonesia today show a very rapid progress by doing research of the utilization of traditional medicine. Medicinal herbs have been popular because it is easier to obtain, the price is quite affordable and minimal side effects compared to chemical drugs<sup>10</sup>.

One traditional medicinal plants now widely used and cultivated into one agricultural commodity is the fennel plant (*Foeniculum vulgare* Mill)<sup>11</sup>.

Fennel fruit is dried fruit, blackish brown and useful for cough medicine, stomach ache, ulcers, throat lozenges. Fennel fruit function as a medicinal plant related to its chemical content consisting of essential oils, flavonoids, saponins, glikosidastilben funikulosida I, II, III, IV, stigmasterin, fatty oils, proteins, organic acids, pentosan, pectin, trigonelin, choline, and iodine<sup>12</sup>.

The ability of 100% fennel fruit extract 100% decrease inflammation in the oral mucosa of Wistar rats have been demonstrated in Andajani and Mahardika research<sup>13</sup>. In addition, Setyaningsih research showed that administration 50% of fennel fruit extract in the gingival wound of

Sprague Dawley rats were able to increase the number of fibroblasts<sup>14</sup>. It was also reported by Mandala that extract of fennel fruit is able to induce reepithelialization in the gingival wound<sup>15</sup>. Based on Anton research fennel extract can decrease PMN leukocytes cells and increase the density of angiogenesis in healing process of labial gingival wound Sprague Dawley rats<sup>16</sup>. A study in 2004 showed that fennel contains components such as anti-inflammatory, analgesic, and antioxidants such as flavonoids, saponins and ascorbic acid that help healing process<sup>17</sup>.

based on that, the researchers are interested to examining the influence of differences effect topically of 50% ethanol fennel fruit extract and Povidone Iodine in enhance angiogenesis and reepithelialization on traumatic ulcer of oral mucosa white male rats.

## **MATERIALS AND METHODS**

The design of the study is experimental research with Randomized Post Test Only Control Group Design<sup>18</sup>.

### **Manufacture of 50% Fennel Ethanol Extract**

Fennel fruit used in this study was obtained from Singaraja Bali. Fennel fruit extract obtained by finely grinding fennel fruit. Then add 70% ethanol stirred for 30 minutes with a magnetic stirrer and leave for 48 hours. The results of maceration filtered 3 times with buchner funnel lined with filter paper and erlenmeyer accommodated. The filtrate was

evaporated with a vacuum rotary evaporator. The extract diluted with aquadest to get 50% of concentration<sup>19</sup>.

### **Treatment in Animal Experiments Prior Research**

1. Thirty-two male white rats, placed in cages, each cage containing three rats. 2. Cage made of plastic container measuring 23cm x 17cm x 9.
2. The cages were placed in a room with good ventilation, sufficient light, quiet, not noisy, the temperature is set at room temperature of 20°C.
3. Rat adapted for 7 days, given water to drink and a standard diet using HPS 594 brands of food production PT Charoen Pokphand.

### **4. During Research**

1. Thirty-two mice that have been adapted got 30% hydrogen peroxide application using cottonbud on labial mucosa oral for two minutes a day and given for 3 days to make traumatic ulcer.
2. To easier application of hydrogen peroxide, each mice anaesthetized using a combination xylazin (5mg / kg) and ketamine (20mg / kg) intraperitoneally<sup>20</sup>.
3. Mice that have been made on the labial mucosa ulceration of the lower lip then divided into a control group (16 mice) and the treatment group (16 mice).
4. treatment given at fourth day, control group get Povidone

Iodine topically application using microbrush (diameter 2mm) for 2 minutes, 3 times a day, for 3 days. The treatment group get 50% of fennel fruit ethanol extract using microbrush (diameter 2 mm) for 2 minutes.

5. On 7<sup>th</sup> day all experimental animals euthanized use of inhaled ether and labial ulceration tissue of the mucosa on lower jaw collected.

### After Research

a.

After euthanasia and removal of tissue, mice used in this study as soon buried and treated as well as possible. Mandibular labial mucosa specimens were taken, fixed with Neutral Buffer Formalin (NBF) 10% to make microscopic preparations. For all specimens, cutting with microtome done with a thickness of 5 microns, taken for Harris Hematoxylin eosin stained. Comparison between groups was performed by microscopic examination by observing neoangiogenesis and reepitelization seen in microscopic preparations and seen in 4 of the visual field using a microscope Olympus CX21 electric brand with 400x magnification.

a. Angiogenesis is determined by counting the number of newly formed blood vessels. Shooting using videophoto with

three repetitions.

b. Reepitelialization determined by measuring the width of the epithelium using morphometric methods with units of micrometers. Shooting using videophoto with three repetitions.

### RESULT

Results of research the differences effect of 50% fennel ethanol extract and Povidone Iodine to increased angiogenesis and reepitelialization for traumatic ulcer on oral mucosa white male rats

**table 5.2 Normality Test Results of neoangiogenesis (units) and reepitelialization (µm) of the oral mucosa of control group and groups of 50% fruit ethanol ethanol extract**

Variable	Groups	p	Description
Neoangiogenesis (unit)	Control	0,325	Normal
		0,608	Normal
Reepitelialization (µm)	50% fennel ethanol extract	0,279	Normal
		0,400	Normal

It can be concluded that the entire group of data have normal distribution ( $p > 0.05$ ). Therefore, testing of the data was performed using the parametric method, in this case using independent test T-test.

table 5.3 Homogeneity Test Results of neoangiogenesis (units) and reepitelialisasi (m) of control groups and 50% of fennel ethanol extract

Variable	F	P	Description
Angiogenesis (unit)	1,488	0,232	Homogen
Reepitelialization (µm)	0,368	0,549	Homogen

All data is presented in Table 5.3 is greater than the value of alpha. It can be concluded that the data variance between groups is homogeneous ( $p > 0.05$ ).

table 5.4 The neoangiogenesis (unit) mean and Comparison Test Results using independent T-test.

Groups	n	Neoangiogenesis (Unit) Mean	SD	Mean Difference	t	P
Control	16	16,5	1,63	-	-	0.000
50% of fennel ethanol extract	16	39,19	2,28	22,68	32,29	0

Table 5.4 shows that the average neoangiogenesis control group was  $16.5 \pm 1.63$  units and the mean treatment group with 50% of fennel ethanol extract was  $39.19 \pm 2.28$  units. Based on these data it can be seen that the average neoangiogenesis is highest in the treatment group administration of ethanol extract of fennel fruit; Analysis of significance with independent test T-test showed that the t value of -32.29

had p-value less than alpha. there is a significant difference of neoangiogenesis between the two compared treatment groups ( $p < 0.05$ ), which means the 50% of fennel ethanol extract increases the angiogenesis more than Povidone Iodine in traumatic ulcer healing on white male rats.

table 5.5 Reepitelialization mean (µm) and Comparison Test Results of independent T-test.

Groups	n	Reepitelialization (µm)	SD	Mean Difference	t	p
Povidone Iodine	16	2031,06	104,70			
50% of fennel ethanol extract	16	976,88	97,82	1054,18	29,42	0.000

Table 5.5 shows that the average reepitelialization Control group (Povidone Iodine) was  $2031.06 \pm 104.70\mu\text{m}$ , mean treatment group with

the 50% of fennel ethanol was  $976.88 \pm 97.82$   $\mu\text{m}$ . Based on these data it can be seen that the average of the gap epithelium width is highest in control group (Povidone Iodine). Analysis of significance with independent test T-test showed that the t value of 29.42 has a p-value less than alpha. There is a significantly difference reepithelialization between the compared two groups ( $p < 0.05$ ), which means the 50% of fennel ethanol extract increases reepithelialization more than Povidone Iodine in traumatic ulcer healing on white male rats.

## DISCUSSION

On 7<sup>th</sup> days There is a significant neoangiogenesis increase in the Group treatment of 50% fennel ethanol extract Compared to control. This means that the traumatic ulcer healing faster in treatment group compared with control groups .

Results of this study was supported by Anton which conducted that the 50% of fennel ethanol extract can improve gingiva wound healing on rat. Observations were made on day 7 showed an increase in blood vessel proliferation and angiogenesis density. Histopathologic features in the study showed significantly different between treatment group and control group<sup>17</sup>.

The increased neoangiogenesis in this study can be caused by the 50% of fennel ethanol has the potential antioxidant and anti-inflammatory that influential in the process of wound healing. The 50% of fennel ethanol extract contain antioxidant flavonoid, that has the potential to prevent free

radicals which can cause oxidative stress occur and inflammatory responses prolonged that can delay the start of the proliferative phase of wound healing<sup>21</sup>. Flavonoids can decrease inflammation and prevent oxidative damage to tissue in the soft tissues of the oral cavity<sup>22</sup>.

Improvement of wound healing process after given fennel extract is also found in the study by Sahane<sup>23</sup>, it can occur due to the effects of flavonoids, which are contained in it, can increase the expression of growth factors required in the process of new blood vessel formation at the wound area. angiogenesis has an important role in the healing of damaged tissue to help distribute nutrients and oxygen. Angiogenesis, regulated by growth factors which work in synergy. VEGF, angiopoietin and TGF- $\beta$  is a major component in the angiogenesis.

Angiogenesis in the control group was lower than treatment group Brinemark et al examined the effects of administration of Povidone Iodine on a small wound in the hamster cheek found the group given Povidone Iodine for one minute stoppage of blood flow occurs at the capillary surface and do not return to normal within an hour<sup>24</sup>. Research Brennan and leaper which examines the microcirculation in the wound granulation tissue in rabbit ears, found that after the granulated wound drained saline and Povidone Iodine, microcirculation examined under a microscope to determine the effects of the material solution on granulation tissue microcirculation, Povidone Iodine group showed cessation blood

flow in tissue granulation and not improved for 72 hours<sup>25</sup>.

To see an increase of reepithelialization, measured of the gap epithelial width that still exists with morphometry methods. Test comparison of the average width of the epithelial gap between groups using independent test T-test, showed wide differences epithelial significant gap in the control group and the treatment group ( $p < 0.05$ ) is presented in Table 5.5. There is a significant increase in reepithelialization on the treatment group of 50% fennel ethanol extract compared to the observation group on 7<sup>th</sup> days. On treatment group, the open epithelial gap is smaller than the control group. This means that the process reepithelialization of traumatic ulcer healing the oral mucosa faster occurs in the group that given 50% of fennel ethanol compared to the control group.

Increased reepithelialization in the group that given 50% of fennel ethanol extract can be caused by the increase of growth factors required in the migration and proliferation of epithelial cells. Fennel fruit showed anti-inflammatory and antioxidant effects that can improve wound healing by increasing co-Transforming Growth Factor-beta 1 (TGF- $\beta$ 1). TGF- $\beta$  1 stimulates the migration and improve adhesion strength epithelial cells<sup>24</sup>, increase the activity of the enzyme, induces the synthesis of nitric oxide and COX-2 that are required in the process of wound reepithelialization<sup>26</sup>.

In this study, there is a significant increase in reepithelialization in the group given 50%. of fennel

ethanol. This shows the same results with research Sahane et al., (2015) which states that fennel fruit extract can increase the regeneration of epithelial cells significantly in the treatment group. Based on these studies, Sahane et al., (2015) concluded that fennel fruit can accelerate wound healing<sup>24</sup>. Increased reepithelialization can be caused because of the potential fennel fruit as antioxidants in preventing the formation of free radicals that inhibit cell migration. Fennel fruit prevent delayed of the proliferative phase of wound healing that caused by free radicals and enhance tissue regeneration to restore its integrity. Fennel fruit also shortens the process of inflammation and regulate cellular responses to wound<sup>27</sup>.

Epithelial gap in control group that given Povidone Iodine wider than the treatment group, it's also found in Kjolseth et al research. The study showed the group given Povidone Iodine require a longer time to epithelialization than the control group that were given saline<sup>28</sup>. Likewise research Gruber et al. (2005) compares the time required to epithelialization in partial and full thickness wound in mice, then given a 3% hydrogen peroxide, Povidone Iodine, acetic acid, saline respectively 4 times a day. The result is no time difference between Povidone Iodine epithelialization and saline<sup>29</sup>.

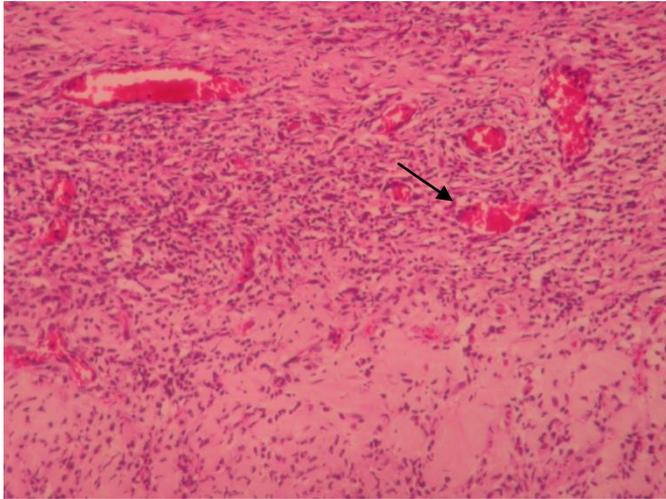
Saponin is triterpena or steroids, especially as there are glycosides that cause a bitter taste of plants<sup>30</sup>. According tirterpen activities can serve as a cytotoxic, cytostatic, antimicrobial,

anti-inflammatory and spermicides and affect the metabolism and biosynthesis<sup>31</sup>. Flavonoids work to improve capillary fragility and can be disinfectant<sup>32</sup>. Flavonoids function as an antiseptic and anti-inflammatory. As anti-inflammatory flavonoids work by suppressing local swelling so that the blood supply to the wound area is not disturbed. Deficiency of blood supply to the injured area causes a delayed in wound healing<sup>33</sup>.

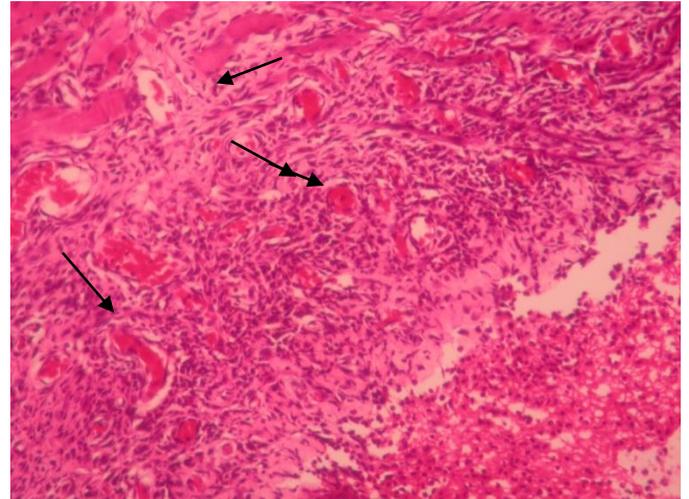
Vitamin C in the wound will increase the formation of hydroxyproline which is one constituent of collagen. The more hydroxyproline the amount of collagen will be more and more formed. Collagen is a protein fiber fibrose which serves to provide strength to the wound to accelerate the closure process of the wound. Furthermore, Parker stated that the administration of vitamin C can increase the activity and the number of fibroblasts. Increasing the number of these cells will stimulate an increase in the number of fiber-fiber collagen, elastic and glycosaminoglycans.

Glycosaminoglycan is a basic substance that serves as a barrier against penetration of bacteria<sup>34</sup>. Fennel fruit (*Foeniculum vulgare* Mill.) that contained vitamin C can accelerate the wound healing process. Vitamin C has an important physiological role for the growth of epithelial skin, protect mucosal and epithelial cells from the keratinization process and increase mucosal resistance to infection by covering epithelium. Topical application of drugs is used so that the

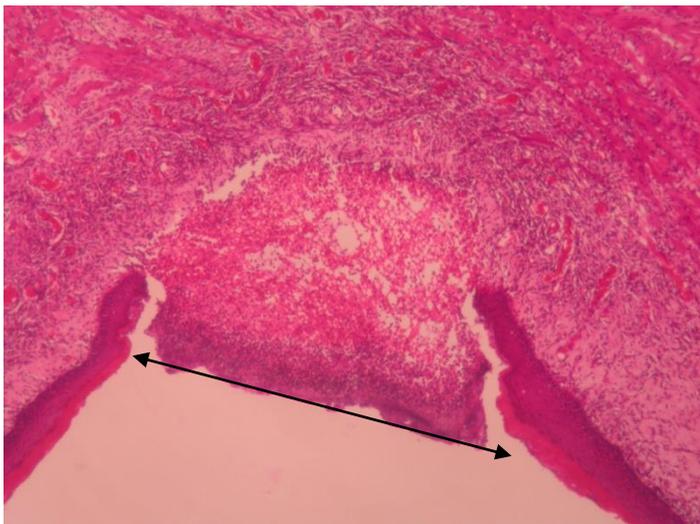
penetration of the drug on the mucosal is quickly and immediately reached the target. Usefulness and efficacy of topical treatment derived from the physical and chemical effects of drugs that are applied above traumatic ulcer on oral mucosa<sup>35</sup>.



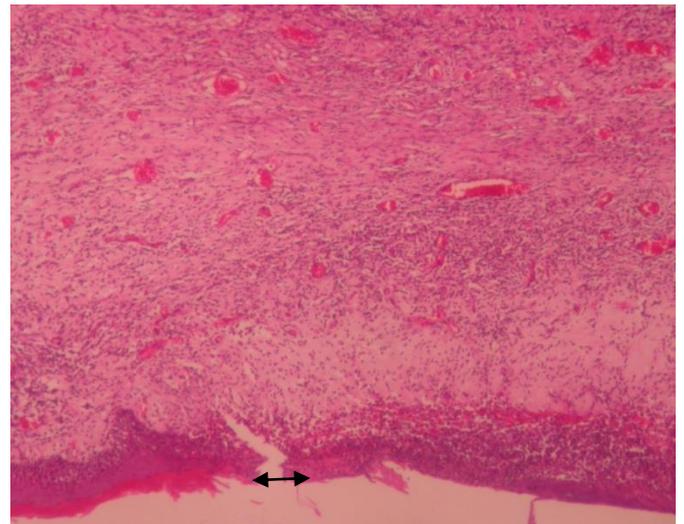
Angiogenesis of Control Group  
(Povidone Iodine)



Angiogenesis of 50% Fennel Etanol Extract  
(*Foenicullum vulgare* Mill)



Reepitelialization of  
Control Group  
(Povidone Iodine)



Reepitelialisasi of 50% Fennel Etanol Extract  
(*Foenicullum vulgare* Mill)

Description: Histological features (Figure A, B, C, D) there are increase angiogenesis and reepitelialization in the treatment group of 50% fennel ethanol extract (*Foenicullum vulgare*) Hematoxylin eosin staining. (Pictures taken with electrically microscope Olympus CX21 magnification 400x).

## CONCLUSION

Based on the research that has been done, it can be concluded that topical administration of 50% fennel ethanol extract more increased angiogenesis and reepithelialization than Povidone Iodine for traumatic ulcer healing at oral mucosa male rat.

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