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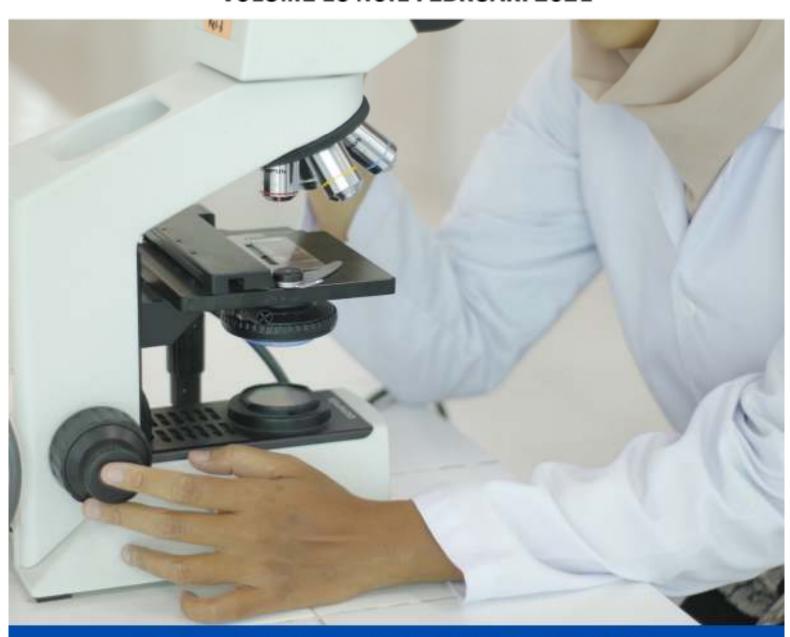


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RESEARCH ARTICLE

Beneficial Effect of Arabica Coffee Fruit Skin (Coffea Arabica) on Epithelial Thickness after Tooth Extraction

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ABSTRACT

Background: In Indonesia, tooth extraction is the most common practice in dentistry. A successful indicator of tooth extraction is a perfect healing process. One of the parameters of a perfect healing process is epithelial formation. Based on previous research, arabica coffee fruit skin has proven potential to accelerate the wound healing process. The compounds in the arabica coffee fruit skin are mostly composed of flavonoids, tannins, and chlorogenic acid. Purpose: This research is proposed to understand the beneficial effect of arabica coffee fruit skin (Coffea arabica) towards the increase of epithelial thickness in post tooth extraction socket. Material and Method: The type of this research was laboratory experimental in vivo with post-test only control group design using 24 Wistar rats as a sample. The sample was separated into two groups, control and intervention groups with 12 Wistar rats each. Treatment in both groups was given during 3, 5, and 7 days. Afterward, tissue processing undergoes with buccolingual cutting and HE coloring. Measurements are carried out with the ImageRaster software in the thickest and thinnest part of the epithelium that covers the tooth socket. Result: Epithelial thickness after tooth extraction increased significantly (p≤0.05) in the intervention group compared to the control group. Conclusion: Arabica coffee fruit skin has the beneficial effect of increasing the epithelial thickness after tooth extraction.

Keywords: Tooth extraction, epithelial thickness, arabica coffee fruit skin

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INTRODUCTION

Tooth extraction is the most common procedure in dentistry. According RISKESDAS 2018, the amount of tooth extraction is higher (7,8-8%) than restoration (6,6-6.8%).¹ Tooth extraction is usually performed when the conservation treatment fails or is not indicated. Extraction of teeth is successful if it is following by an adequate process of healing.²

Sometimes, the healing process causes a problem that leads to failure of wound healing. The most common problem is dry socket,³ with an incidence of 5% of all failure wound healing cases after tooth extraction.⁴ Dry sockets occur as a result due to the disintegration of blood clots by fibrinolysis⁴ which causes the healing process to be interrupted. Therefore, the wound healing process needs to be considered to avoid the presence of a dry socket.

There are three stages of the wound healing process after tooth extraction, are inflammation stage, proliferation, and remodeling.^{5,6} The proliferation stage is one of the most important stages in the wound healing process. In the proliferation stage, the epithelial tissue damaged will undergo а reprocess. epithelialization the ln reepithelialization stage epithelial cells migrate, mitosis, and differentiation. This stage will restore the lost mucosal integrity.7 The formation of the epithelium is a parameter of the success of wound healing after tooth extraction. When the epithelialization process does not occur, the wound cannot be healed.8

The wound healing process can be done, one of which involves administering traditional natural medicines. According to Riskesdas 2018, 31.5% of Indonesia's population uses traditional health treatment to treat their disease disorders. Nationally, the population of East Java is the area that uses medicinal herbs the most (48.3%). Traditional medicine treatments made from nature are preferred because they are considered cheaper with fewer side effects. 9

One of the compounds from natural ingredients that influence the wound healing process is arabica coffee fruit skin. The arabica coffee fruit skin is the main by-product (waste) generated from coffee processing.¹⁰ Based on the previous research, showed that the content of arabica coffee fruit skin has proven potential in increasing the number of fibroblast cells in wounds after tooth extraction. 11 Fibroblasts are cells that play an important role in wound healing because they are responsible during the tissue reconstruction process.⁶ Compounds in the arabica coffee fruit skin are mostly composed of flavonoids. condensed tannins (proanthocyanidin),12 and chlorogenic acid13 that play an important role as antioxidants, antiinflammatory, and antibacterial¹⁴ which can accelerate the wound healing process.

According to the above explanation, it could be interesting to understand the beneficial effect of arabica coffee fruit skin (*Coffea arabica*) towards the increase of epithelial thickness in the post tooth extraction socket of Wistar rat.

MATERIALS AND METHODS

This laboratory experiment in vivo with post-test only control group design was approved by the ethical committee of the medical research faculty of dentistry Universitas Jember (No.988/UN25.8/KEPK/DL/2020). The sample of this research was 24 male Wistar rats separated into two groups named control and intervention group. The control group was given a solution of 2 ml water. Meanwhile, the intervention group was given a solution of powdered arabica coffee fruit skin with the dose of 0.117 gr dissolved in 3.6 ml warm water. Both treatments were given intragastrically during 3, 5, and 7 days. Then, Wistar rats decapitated 24 hours after the last treatment on days 4, 6, and execute tissue processing buccolingual cutting section and Hematoxylin-Eosin (HE) coloring.

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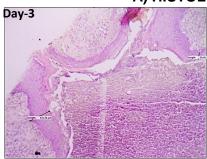
Next, the epithelium of the tooth socket was observed using OptiLab that was connected with a binocular microscope with a 100x magnifier. Measurements are carried out with the help of the ImageRaster 3.0 software in the thickest and thinnest part of the epithelium that covers the tooth socket. Data then tested using a One-Way ANOVA test with a 95% confidence level. Afterward, tested with post hoc Least

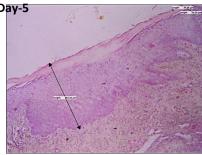
Significant Difference test to see differences between each group.

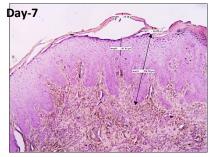
RESULT

Based on the research that has been done, it was found that the average of epithelial thickness in the control and intervention group from days 3, 5 and 7 were increased (Figure 1).

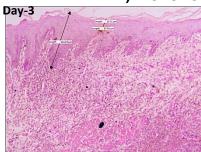
A) HISTOLOGICAL FEATURES OF CONTROL GROUP

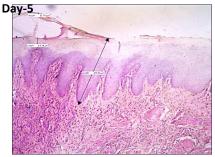






B) HISTOLOGICAL FEATURES OF INTERVENTION GROUP





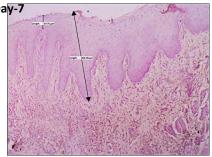


Figure 1. The gingival epithelium covering the tooth socket with 100x magnification. A) Control group show the epithelial formation process that occurs physiologically, starting from the socket which is still open on the 3rd day, until it is closed on the 5th day, and has increased in thickness on the 7th day; B) The intervention group showed a process of increasing the thickness of the epithelium from the 3rd, 5th, and 7th day due to the effect of the powdered arabica coffee fruit skin.

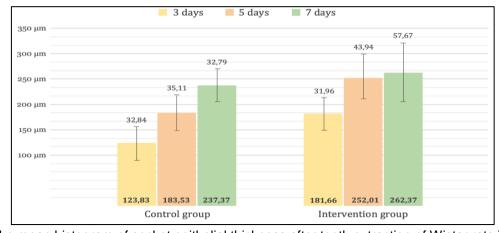


Figure 2. The mean histogram of socket epithelial thickness after tooth extraction of Wistar rats in the control group and the intervention group was accompanied by standard deviations.

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The results showed that the average epithelial thickness after tooth extraction in the control group from the 3rd, 5th, and 7th days is increased. Likewise, the intervention group that was given a solution of powdered arabica coffee fruit skin, from the 3rd, 5th, and 7th days also experienced an increase. The control group experienced a stable increase in epithelial thickness. Meanwhile, in the intervention group, a significant increase in epithelial thickness was only seen between day 3 and day 5, then between day 5 and day 7 did not show a significant increase.

When compared, the mean epithelial thickness of the intervention group on day 3 was almost the same as the mean epithelial thickness of the control group on day 5.

One-Way ANOVA test results obtained a significance value of 0.01 ($p \le 0.05$), which means that there is a significant difference in the average epithelial thickness between all groups. Furthermore, to find out which groups have significant differences in between can be done by LSD Test as summarized in Table 1.

Table 1. Summary of data analysis epithelial thickness after tooth extraction between each group

| GROUP | n | Epithelial Thickness (μm) | | | n |
|--------------|---|-------------------------------------|-------------------------------------|-------------------------------------|--------|
| | | DAY-3 | DAY-5 | DAY-7 |] P |
| Control | 4 | 123.83 <u>+</u> 32.84 ^{aA} | 183.53 <u>+</u> 35.11 ^{aB} | 237.37 <u>+</u> 32.79 ^{aB} | 0.001* |
| Intervention | 4 | 181.66 <u>+</u> 31.96 ^{aA} | 252.01 <u>+</u> 43.94 ^{bB} | 262.37 <u>+</u> 57.67 ^{aB} | 0.001* |
| р | | 0.057 | 0.023* | 0.390 | |

The value was expressed as mean $\underline{+}$ standard deviation ($\bar{X}\underline{+}SD$)

Explanation:

(*) = there was a significant difference ($p \le 0.05$)

(ab) = difference superscript in a column shows the significant difference

(AB)= difference superscript in a row shows a significant difference

Based on the results of the LSD test, shows that there is a significant difference between each group. In the control and intervention groups, there was a significant difference ($p \le 0.05$) between day 3 and day 5. Meanwhile, between day 5 and day 7 in both the control and intervention groups, there was no significant difference.

DISCUSSION

Tooth extraction action can cause injury of the tissue around the tooth. According to Ningsih et al.¹⁵, after an injury occurs, the socket will be healing physiologically. This healing process involved soft tissue and hard tissue. The blood clot is formed, which will develop into granulation tissue in soft tissue. Furthermore, the epithelium will be developed to cover the surface of the granulation tissue completely.

Epithelialization is an essential component of wound healing used as a defining parameter of

successful wound closure.⁸ The faster the reepithelialization was done, the faster the wound will be closed, so the faster the wound healing will occur.¹⁶

Based on the previous study, the mean epithelial thickness in the control group on days 3, 5, and 7 has increased. Based on the theory, the wound healing response occurs physiologically. However, statistically significant differences, based on the post-hoc test with LSD, only existed between day 3 and day 5 (p<0.05). This shows that the epithelialization process has increased significantly. This increase can be seen histologically, the state of the socket that is still open to being closed by the epithelium. Smith¹⁷ reported on the 2nd day after tooth extraction in mice, epithelial migration has begun physiologically, and on the 4th day, epithelial migration has increased. This situation causes a significant increase in epithelialization. Next, on observations between days 5 and 7, although there was a stable mean increase in epithelial

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thickness, it was not significant in statistics (p>0.05). These results show, there is no difference between the mean epithelial thickness at day 5 and day 7. Supported by Smith¹⁷, on the 5th day after tooth extraction in mice, the epithelium almost covers the socket and the trabeculae of the cartilage have adhered to the wall at the base of the socket physiologically. Then, on day 8, epithelialization was complete and the 1/3 apical of the socket was filled with cartilage and osteoid. This shows that the physiological proliferation phase of the wound healing process has been completed, and the initial bone formation process begins.

In the intervention group, the mean epithelial thickness on days 3, 5, and 7 also increased. Similar to the control group, there were significant differences, based on the posthoc test with LSD, which only existed between day 3 and day 5 (p<0.05). Meanwhile, between days 5 and 7, the epithelialization process was almost complete because it did not experience a significant increase and almost had the same thickness mean. This was supported statistically, which showed there was no significance (p>0.05). It means, there is no difference between the mean epithelial thickness at day 5 and day 7. It can also be seen histologically that the epithelial thickness at day 5 and day 7 does not appear to have a significant difference in thickness. This may be caused by the epithelialization process which is almost over. It has been known previously that epithelial cell migration will stop if the epithelial cells have made contact with other epithelial cells in all directions (contact inhibition).15 Thus, it can be concluded that on the 5th and 7th day, by giving a solution of powdered arabica coffee fruit skin, the epithelial cells in the intervention group have reached an almost complete migration pattern. According to Throne⁶, after the migration of epithelial cells (keratinocytes) has completed, the remodeling process in the socket begins.

The mean epithelial thickness of the treatment group on days 3, 5, and 7, when compared, was higher than the mean epithelial

thickness of the control group. This shows that, by giving a solution of powdered arabica coffee fruit skin affects increasing the thickness of the epithelium in the wound healing process after tooth extraction of Wistar rats.

The increase in epithelial thickness that occurred in the intervention group was caused by the potential content of chemical compounds in the skin of the Arabica coffee fruit. Arabica coffee fruit skin contains polyphenols which are composed mostly of flavonoids and condensed tannins (proanthocyanidin)¹², and also phenolic acids with the highest content in the form of chlorogenic acid.¹³ These three compounds have an important role in accelerating wound healing.

Flavonoids are known to play an important role in wound contraction and increase the rate of re-epithelialization. In a study by Thakur et al. 19, it was explained that flavonoids play a role in the wound healing process because of their astringent and antimicrobial properties which are responsible for wound contraction and an increase in the rate of epithelialization. Flavonoids also possess immunostimulating effects to increase phagocytosis activation by macrophages and increase the inflammatory process. The increase in macrophages affects the production of growth factors that contribute to the proliferation process, thereby helping to accelerate the re-epithelialization process⁷. Also, flavonoids are powerful antioxidants that can reduce lipid peroxidase and increase the speed of epithelialization.¹⁶ The reduction of lipid peroxidase from flavonoids will prevent necrosis and increase the strength of the collagen fiber webbing.¹⁸

The other compound content is tannins. The active substance of tannins can play a role in increasing wound contractions and the speed of epithelialization. Tannins as an antibacterial can reduce inflammation, increase epithelial formation, and cause a vasoconstrictive effect on blood vessels. Tannins are thought to play a role in regulating transcription and translation of vascular endothelial growth factor (VEGF). VEGF acts paracrine not only on skin vascular endothelial cells but also on keratinocytes and

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immune cells which promote re-epithelialization and at the same time stimulate angiogenesis and restore oxygen perfusion.8 In addition, tannins have an anti-inflammatory effect that increases the proliferative ability of TGF-ß to increase the epithelialization process. TGF-ß acts as an important regulator in returning activated keratinocytes to the basal cell phenotype. Its expression during injury has been shown to granulation tissue induce formation myofibroblast differentiation which facilitates contraction of the collagen matrix and wound closure.8 This was evidenced in the study of Rahayu et al.²¹, regarding the effectiveness of proanthocyanidin (condensed tannin) of cocoa pod skin against the socket epithelium after tooth extraction of Wistar rats, which showed an increase in epithelial thickness. Condensed tannins also act as antioxidants which have a role in accelerating the healing process.

The other chemical compound is chlorogenic acid. The amount of chlorogenic acid found in coffee reaches 90% of the total phenol.²² Chlorogenic acid has strong antioxidant activity as well as anti-inflammatory activity. 10 Duangiai et al.23 in their research stated that the content of chlorogenic acid and caffeine in the skin of arabica coffee fruit plays a role in antimicrobial activity. Duangjai's research shows that Grampositive bacteria are more susceptible to the skin of arabica coffee fruit than Gram-negative bacteria. Its effectiveness on Gram positive and negative bacteria is influenced by the structure of the bacterial envelope and active compounds from the arabica coffee fruit skin.

In this study, potential compounds in the arabica coffee fruit skin were shown to increase the thickness of the socket epithelium after tooth extraction. The role of arabica coffee fruit skin compounds in increasing the thickness of the epithelium is seen at the beginning of wound healing. An increase in epithelial thickness in the intervention group was seen on day 3. The mean epithelial thickness in the intervention group on day 3 was almost the same as the mean epithelial thickness in the control group on day 5. This was proven statistically that there was no significance

(p>0.05). It means, the epithelial thickness of the control group on day 5 was reached in the intervention group on day 3, and the two means were proven to have no significant difference. This shows, by giving a solution of the powdered arabica coffee fruit skin, epithelialization increases in the early stages of wound healing, thus indicating a faster wound healing process. It was also seen that the epithelial thickness in the intervention group on day 3 was almost the same as the epithelial thickness in the control group on day 5 histologically, and the sockets of the two groups had both been closed by the epithelium. Although they had almost the same mean epithelial thickness, the epithelium in the intervention group on day 3 had a clearer rete peg shape than that in the control group on day 5. The existence of a rete peg indicates that the re-epithelialization process goes well informing the epithelial tissue back to normal.⁵ So, apart from being able to increase the thickness of the epithelium, giving a solution of powdered arabica coffee fruit skin is proven to produce a better reepithelialization process.

CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that the compound content of arabica coffee fruit skin (Coffea arabica) has the beneficial effect to increase the epithelial thickness of Wistar rat after tooth extraction.

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