The effect of *Persea americana* Mill. seed extract on inflammatory cells and fibroblast formation in tooth extraction socket healing

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**ABSTRACT**

**Background:** Inflammatory cells and fibroblasts have an essential role in the wound healing process. *Persea americana* Mill. seed categorises as a waste; it contains rich nutrients that can accelerate wound healing activity. **Purpose:** This study aims to determine the effect of *Persea americana* Mill. seed against inflammatory cells and fibroblast formation in tooth extraction socket healing. **Methods:** Ninety-six Sprague Dawley rats had their lower left molars removed. Forty-eight rats tested for inflammatory cells were divided into four groups: negative control group (IC1), positive control group (IC2), *Persea americana* Mill. seed extract concentrations of 50% (IE1) and 90% (IE2). Another 48 rats used for fibroblast were divided into three groups: the control group (FC1), *Persea americana* Mill. seed concentrations of 50% (FE1) and 90% (FE2). The gel was applied to the socket under general anaesthesia. Four rats from each group were decapitated for histopathological tissue preparations with Haematoxylin Eosin (HE) staining on the 3rd, 5th and 7th days for inflammatory cells and the 3rd, 5th, 7th and 14th days for fibroblast formation. The preparations for each research were scored under the microscope at 40x magnification. The obtained data was analysed using the Kruska—Wallis and the Mann—Whitney test. **Results:** A significant decrease (p<0.05) of inflammatory cells in IE2 on the 5th and 7th day. A significant increase (p<0.05) of fibroblast formation between treatment and control groups and no significant difference (p>0.05) between FE1 and FE2 was based on the interval days. **Conclusion:** *Persea americana* Mill. seed extract can decrease the inflammatory cells and accelerate the fibroblast formation in tooth extraction socket healing.

**Keywords:** fibroblast; inflammatory cells; *Persea americana* Mill.; tooth extraction socket healing

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**INTRODUCTION**

Tooth extraction is one of the most frequent oral surgical procedures and can lead to post-extraction complications such as pain, swelling, infection and mastication problems. The tooth extraction socket heals when the surface closes and when the tissue strength is normal. The tooth extraction process will damage the hard and soft tissue, and physiologically, the body will heal the wound. Wound healing in post-dental extraction is a complex process to restore the epithelial regeneration and the formation of connective tissue in the general principle of wound healing. Wound healing is divided into four phases: the haemostasis phase, the inflammation phase, the proliferation phase and the maturation phase.

In the early stage of the inflammation phase, the first inflammatory cell chemically attracted is the neutrophil in polymorphonuclear (PMN) cells. Neutrophils have the function of attacking and destroying invading microorganisms during blood circulation in the early stage of the inflammatory process. Neutrophils appear in large numbers during the first days of inflammation. Many neutrophils are present due to the infiltration and accumulation of leukocytes from the blood vessels into the injury site. After 24–48 hours, the neutrophil cells transition into macrophages. Macrophages can phagocytise microorganisms and also secrete growth factors such as fibroblast growth factors (FGF). The formation of fibroblasts plays an essential role in the wound healing process, and fibroblasts can fuse the
wound edges together, bringing them closer and attached. Fibroblasts migrate to the wound site and proliferate, resulting in a predominance of their numbers at the wound site. Fibroblasts first significantly create on day three and reach their peak on day seven. Another study stated that the fibroblast formation starts to decrease to normal levels by around day fourteen. Fibroblasts infiltrate and degrade the fibrin clot by producing extracellular matrix components. This matrix complex supports and regulates fibroblast migration and activity, as well as granulation tissue generation and epithelialisation.

Most of the world’s population began to reintroduce the concept of natural life, where natural ingredients are used again, including treatment with medicinal plants. Based on data from World Health Organization, about 80% of the world’s population uses medicinal plants as alternative medicine. Persea americana Mill. is a plant widely grown in a tropical country and is one of the medicinal plants in various health fields because it contains essential bioactive ingredients. In addition, it is also known for its fruit, leaves and seeds having multiple benefits and high nutritional content.

The seeds of the fruit are non-edible and categorised as wastes. The seeds contain several secondary metabolite compounds based on phytochemical screening, namely alkaloids, triterpenoids, tannins, flavonoids and saponins. These secondary metabolite compounds, such as tannins, flavonoids and saponins, can stimulate the migration and formation of fibroblasts in the wound area. Other studies have also shown that Persea americana Mill. seed extract is a rich source of oleic acid and contains essential fatty acids. When used in pharmaceutical formulations for topical use, it can decrease inflammatory cells during wound healing. Thus, it can be considered an option to treat a wound.

This study aims to determine the effect of Persea americana Mill. seed extract against inflammatory cells and fibroblast formation in tooth extraction healing in Sprague Dawley rats.

MATERIALS AND METHODS

The research is an in vivo experimental laboratory with a randomised controlled and post-test control group design. The ethical clearance was approved by the Dentistry Ethics Committee in Universitas Trisakti (letters no. 347/KE/ FKG/8/2016 and 385/KE/FKG/11/2016). The research was conducted from October 2016–January 2017. The material extraction of Persea americana Mill. seed was conducted at Balai Penelitian Tanaman Rempah dan Obat (Balittr), Cimanggu, Bogor, V-stem Laboratory, Bogor District, Indonesia, and the research with Sprague Dawley rats was conducted at Pertanian Bogor Institute (IPB), Babakan, Bogor, Indonesia.

Fresh Persea americana Mill. seeds were obtained from plantation cultivation in Bangka Belitung, Indonesia, then were washed thoroughly and dried in an oven for 24 hours. After drying, the Persea americana Mill. seeds were cut into small pieces and made into powder, then extracted with ethanol 96% concentrations for 30 minutes. The extracts continued with the maceration process for 24 hours and then were filtered with a Buchner funnel (Fisherbrand, New Hampshire, USA). The obtained filtrate was evaporated with a rotary evaporator (Buchi R-110, Flawil, Switzerland) at a temperature of 40°C and given vacuum pressure to obtain a thick extract until it did not drip. Persea Americana Mill. seed gel extract was made by mixing the thick extract of Persea Americana Mill. seeds into 1% sodium carboxymethyl cellulose (CMC-Na) solution (Wealthy, Jiangsu, China) as a gelling agent.

The research samples used were 96 white male Sprague Dawley rats, age 2–3 months. The condition of the rats was always monitored so that their food and drinking needs were filled. The samples were divided equally for inflammatory cells and fibroblast formation. There were 48 rats used for inflammatory cell research that were randomly divided into four groups (n=12). Group I was the negative control group (IC1), which did not get any treatment on the socket. Group II was the positive control group (IC2), which was treated with povidone-iodine. Group III was the treatment group administered with Persea americana Mill. seed extract concentration of 50% (IE1). Group IV was the treatment group administered with Persea americana Mill. seed extract concentration of 90% (IE2). Another 48 rats used for fibroblast formation research were divided into three groups (n=16). Group I was the control group (FC1), which did not get any treatment on the socket. Group II was the treatment group with Persea americana Mill. seed extract concentration of 50% (FE1). Group III was the treatment group with Persea americana Mill. seed extract concentration of 90% (FE2).

The lower left molars of the samples were extracted with forceps under general anaesthesia with ketamine 50–80mg/kg (Kepro B.V., Deventer, Netherlands) and xylazine 20mg/kg (Agrovet Market, Lima, Peru) infiltration followed with the topical gel application of the Persea americana Mill. seed extract on the socket for one minute using a plastic instrument. The IE1 and IE2 were given the extracted gel every day, while the IC2 was given povidone-iodine. The same tooth extraction procedure and topical gel application of the Persea americana Mill. seed extract were applied every day for FE1 and FE2.

Tissue preparations were made with decapitating four rats from each group under general anaesthesia with ketamine-xylazine infiltration on the 3rd, 5th, and 7th days for inflammatory cells and the 3rd, 5th, 7th and 14th days for fibroblast formation. In each day interval, the left mandible was cut to the size of a tooth socket fixated with 10% formalin and decalified to be used as a tissue sample for Haematoxylin Eosin (HE) staining. The sample slides were read and assessed under a light microscope (Nikon E-100, Tokyo, Japan) per five-micrometre fields of view on a binocular light microscope with 40x magnification. Each preparation was assessed using a scoring system by
two pathologists. The data obtained was analysed using the Statistical Package for the Social Sciences (SPSS version 20.0, IBM, New York, USA) program with Kruskal–Wallis and Mann–Whitney testing.

RESULTS

The histopathological examination showed inflammatory cells on the 3rd, 5th and 7th days in IC1, IC2, IE1 and IE2. The inflammatory cells were seen the most on day three in all groups; they decreased on day five and were at the lowest level on day seven. Based on the interval of days in the research, the IE2 preparation showed a notable decrease in inflammatory cells on day three and day seven. Figure 1 shows that inflammatory cells in IE2 (yellow arrows) were widely spread on day three and decreased on day seven.

The groups with the highest average score on the 3rd day are IC2 and IE2, while the lowest average scores are IC1 and IE1. On the 5th day, IE2 started to decrease, and the other groups remained the same. On the 7th day, every group had decreased with IC1 having the highest average score while IE1 and IE2 had the lowest average score in inflammatory cells (Figure 2).

Figure 1. Histopathological appearance shows the inflammatory cells (yellow arrow) in IE2 were widely spread on day three (A) and decreased on day seven (B).

Figure 2. The average number of inflammatory cells on the 3rd, 5th and 7th day in each experimental group.

Figure 3. Histopathological appearance shows a significant increase in fibroblast formation (red arrow) of FE2 on the 14th day.
The Kruskal–Wallis test concluded there was no significant difference (p>0.05) for inflammatory cell number in all four groups (IC1, IC2, IE1 and IE2). Kruskal–Wallis test results in IE2 showed a significant difference (p<0.05) in every interval of days (3rd, 5th and 7th day), with the highest average of inflammatory cells on the 3rd day. Using the Mann–Whitney test, the data showed a significant difference (p<0.05) in inflammatory cell decrease on the 5th and 7th day in IE2.

The histopathology showed fibroblast formation was not visible in FC1 and FE1 on the 3rd day, while the fibroblast formation had been formed from the 3rd day until the 14th day in FE2; the fibroblast formation was seen the most widely spread on the 14th day. Fibroblast formation in FC1 was less noticeable than FE1 and FE2, while the comparison of fibroblast formation between FE1 and FE2 in the interval of the day was not much different. The highest increase in fibroblast formation, pointed out by the red arrows, in FE2 was seen on the 14th day (Figure 3).

The fibroblast formation average score on the 3rd day was only seen in FE2, while FC1 and FE1 were not formed yet. Fibroblasts have formed in all groups on the 5th day with the highest FE2. Fibroblast formation rapidly increased on the 7th day in every group, and on the 14th day, fibroblast formation still increased with FE2 as the highest, followed by FE1 and FC1 as the lowest (Figure 4).

The Kruskal–Wallis test concluded that there was a significant difference (p<0.05) in fibroblast formation between the control group (FC1) and the treatment groups (FE1 and FE2). The data continued with the Mann–Whitney test; no significant difference (p>0.05) in fibroblast formation in FE1 and FE2 based on day interval.

**DISCUSSION**

This research showed the highest number of inflammatory cells in the control group, and the treatment group was on the 3rd day. The inflammatory cells in IC1 and IC2 did not decrease on day five. The decrease occurred on day seven but was not significant. There was no decrease in IE1 on day five but a significant decrease on day seven, while in the IE2 group, there was a significant decrease on day five and day seven. It shows that Persea americana Mill. seed is rich in nutrients, and secondary polyphenol compounds are beneficial for antioxidants, are anti-inflammatory and promote wound healing activities. The previous research also found the use of oleic acid in Persea americana Mill. can promote a reduction in the number of inflammatory cells in the injured tissue.

This research proves that IE2 is more effective at decreasing inflammatory cells than IE1, as the content in IE1 was not adequate for reducing inflammatory cells. This proves that the greater the concentration of Persea americana Mill. extract, the higher its effectiveness in reducing inflammatory cells. This analysis is in line with the previous research regarding the effects of Foeniculum vulgare Mill. extract on the healing of labial gingiva mucosal wounds, as it was stated that the concentration of plant extracts is too low to contain chemically active compounds, so the biological function is not optimal.

The time interval in this research began on day three because it is based on the theory that states that the proliferative phase postoperatively begins on day three to week three. Histopathology examination showed that fibroblast formation had formed on day three of FE2. However, histopathology showed fibroblast formation was not visible in FC1 and FE1 on day three. This condition is related to the incomplete or nonoptimal number of fibroblast cell mediators in the wound healing process.

The highest fibroblast formation was seen on day fourteen in all groups. It proves that even without therapy, physiologically, the number of fibroblasts will increase from day three to day seven, with the number remaining high on day fourteen. However, in the treatment groups, fibroblasts physiologically increased faster with the support of Persea americana Mill. seed extract. The results align with the previous study, and fibroblasts will appear in the wound area after three days, with the number of fibroblast formations peaking on the seventh day after trauma. However, although the fibroblasts were already high on day seven, they continued to increase until day fourteen.

Fibroblast formation occurred faster in the treatment group (FE1 and FE2) than in the control group. The increase
in the average number of fibroblasts in the treatment group was due to the effect of the *Persea americana Mill.* seed extract. These results are in line with the theory that the increase in the number of fibroblast cells in the treatment group was influenced by the provision of nutrients obtained from the *Persea americana Mill.* seed extract that is useful in accelerating the wound healing process by increasing the process of fibroblast formation.¹⁹

Statistically, there was no significant difference in fibroblast formation in FE1 and FE2 based on day intervals. However, the average fibroblast formation in FE2 was higher than in FE1 based on the day result. This is because FE2 contains more *Persea americana Mill.* seed extract than FE1. The increase of fibroblast formation in the extract group was influenced by the provision of nutrients such as flavonoids, tannins and saponin obtained from *Persea americana Mill.* seed extract.¹⁸ Flavonoids help reduce the duration of the inflammatory reaction, enhance growth factor proliferation and result in fibroblast formation. Tannins can promote the formation of monocytes and macrophages, which secrete growth factors and stimulate the migration and proliferation of fibroblasts in the wound area, accelerating wound healing by increasing the process of fibroblast formation.¹²¹⁸ This research concluded that *Persea americana Mill.* seed extract can decrease the inflammatory cells and accelerate the fibroblast formation. *Persea americana Mill.* seed extract concentration of 90% is more effective than 50% for decreasing inflammatory cells and accelerating fibroblast formation in tooth extraction socket healing in Sprague Dawley rats.

**ACKNOWLEDGEMENT**

The authors are thankful to the Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia for facilitating the research.

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