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THE POTENTIAL OF CACAO (THEOBROMA CACAO L.) POD HUSK EXTRACT ON THE NUMBER OF FIBROBLAST CELLS OF WISTAR RATS WOUND POST-TOOTH EXTRACTION

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ABSTRACT

Objective: The aim of this study was to determine the effect of cacao (Theobroma cacao L.) pod husk extract on the number of fibroblast cells in rats' wound post-tooth extraction.

Methods: A total of 24 rats were divided into 2 groups. Control group and a treatment group. The tooth extraction was performed on a mandibular left first molar. Cacao (Theobroma cacao L.) pod husk extract was applied on the treatment group, and a saline solution was applied on the control group once a day by intragastric administration. On the 3rd, 7th and 10th day, 4 rats' were sacrificed and the mandibulars were subsequently removed for histological process. The fibroblast cells calculation was performed on the histological preparation using a microscope. The data were analyzed using One Way ANOVA.

Results: More fibroblast cells were observed in the treatment group when compared to the control group. The cacao (Theobroma cacao L.) pod husk extract administration for 10 d induced more fibroblast cell quantity compared to that for 3 d.

Conclusion: The cacao (Theobroma cacao L.) pod husk extract administration may enhance the fibroblast cell quantity in the rats with post-tooth extraction wounds.

Keywords: Cacao pods husk, Fibroblast, Tooth extraction

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INTRODUCTION

The healing of post-tooth extraction is a complex and dynamic reaction, the process consists of the inflammatory phase, fibroblastic phase and remodelling phase [1-3].

In the fibroblastic phase, fibroblast cells play a role in the wound healing process. In this phase the angiogenesis process occurs and subsequently forms new blood vessels and proliferation of fibroblast cells that will synthesize collagen, reticular and elastin [4]. The fibroblast cells in wound healing will be affected by growth factors that may induce cell division to produce new cells e. g. PDGF (platelet growth factor), FGF (fibroblast growth factor) and TGF-β (transforming growth factor) inflammatory cells [5].

One of the plants that have potential as an anti-inflammatory is cacao plant (Theobroma cacao L.) that is widely found in Southeast Asia, including Indonesia. In Indonesia cacao production is growing rapidly up to 16 tons as a result it becomes an export commodity that continues to rise from 1990-2005 [6]. Cacao processing also produces waste i.e. cacao pod husks for about 74% of the total fruit. Many studies have been conducted on cacao pod husk main content of which is flavonoids that play a role in anti-inflammatory. Flavonoids in cacao pod husk function to inhibit the expression of Interleukin-2 (IL-2) and Interleukin-1β (IL-1β) in the inflammatory process and may increase TGF-β (transforming growth factor) in order to accelerate wound healing [7].

Previous studies show that cacao pod husk may increase collagen fibres in wound healing of experimental animal gingiva [8]. In other studies, the administration of fresh cacao pod husk extract has anti-inflammatory activity against macrophage cell numbers and increase fibroblast cells by15% of an effective dosage administered in periodontal dressing. The study showed reduced macrophage cells in injured tissue [9, 10].

The purpose of this study was to the observed effect of cacao (Theobroma cacao L.) pod husk extract on the number of wound fibroblast cells of male Wistar rats post-tooth extraction.

MATERIALS AND METHODS

Material

Preparation of cacao (Theobroma cacao L.) pod husk extract was conducted by shredding fresh cacao pod husks and blending it into a fine powder. The fine powder was subsequently macerated using an acetone/aquades solvent a ratio of 7: 3. The simplicia powder was submerged in the solvent in a 1: 2 ratio, stirred and filtered. Maceration was conducted three times. Afterward, the obtained filtrate was evaporated using a rotary evaporator until no solvent was left and the liquid extract was obtained. The liquid extract was concentrated in an oven at 60 °C. To obtain 15% of concentration, 15% of pure extract was dissolved in 85% solvent.

The observation and calculation of fibroblasts were conducted using OptiLab connected to a binocular microscope. The calculation was carried out by 3 observers by counting three different views i.e. on the 1/3 apical of the tooth sockets post-extraction using raster image software with 400X magnification.

The data obtained were subsequently analyzed based on normality test using Shapiro-Wilk test, and Levene test for homogeneity. The data were subsequently analyzed using One Way ANOVA, and continued by LSD (Least Significance Difference).

The study consisted of a control group and a treatment group divided into three subgroups based on the treatment days. Each group had 4 samples, extraction of lower first left molar were performed on all samples under anaesthesia. In the control group, the animals were administered with intragastricsaline solution, while in the treatment group the animals were administered with 15% concentration of cacao pod husk extract intragastrically and were decapitated on the 3rd, 7th and 10th days.

The jaws were first decalcified using formic acid for 7 d, and cut the required part i.e. mesial socket up to the third molar followed by the dehydration process using a multilevel alcohol concentration, clearing using xylol, impregnation, embedded into paraffin. Tissue cutting was performed using a microtome with the thickness of 6μm, placed on a glass object, dried using a warmer slide and painted using Haematoxilin and Eosin stain.

The number of fibroblast cells was calculated under a binocular microscope with 400X magnification. The number of fibroblast cells was histologically calculated on the 1/3 apical buccal, apical and lingual parts with a V pattern on the tooth socket post-extraction.

RESULTS AND DISCUSSION

Based on the study conducted, the average number of fibroblasts of tooth sockets cells in the treatment and control groups is shown in the histogram fig. as shown in fig. 1.

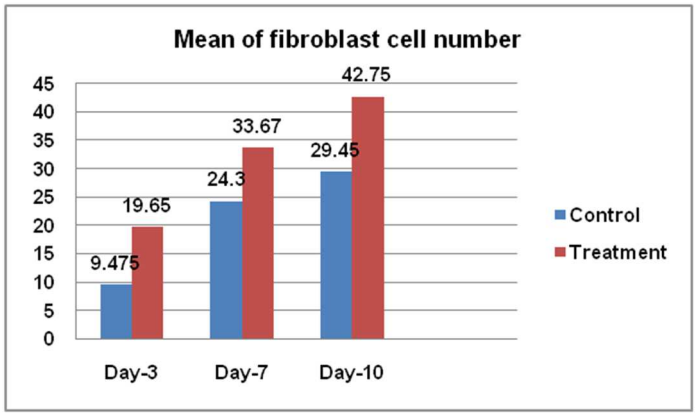

Fig. 1: Histogram of the average number of cells in the treatment and control groups on the 3rd, 7th and 10th days of post-extraction

The data from the observation of fibroblast cell number in male Wistar rats before statistically analyzed were first tested for normality by using Shapiro-Wilk to know whether the subjects of the study were normally distributed. Furthermore, the data homogeneity test was performed using Levene test to know homogeneous data with significant value (p)>0.05.

The data of the research have fulfilled the normality and homogeneity requirement thus it may be continued to perform one-way ANOVA parametric statistic test to determine the difference of an average number of fibroblast cells between treatment groups at 3rd, 7th and 10th-day observations.

The results of One Way ANOVA test result showed that p = 0.00 showing significant differences in the number of fibroblast cells in the treatment group and control groups on the 3rd, 7th and 10th day observations. The data were subsequently tested using LSD to determine the significance differences in each of treatment and control groups. The data resulted from LSD test showed p<0.005 in the comparison of all groups indicating a significant difference in the calculation of an average number of fibroblast cells between the control group and the treatment group on the 3rd, 7th and 10th-day observations.

Cacao pod husk has potential as an anti-inflammatory, anti-bacterial and antioxidant natural medicine [11]. Natural content in the cacao pod husk is a flavonoid that can suppress the inflammatory process. Flavonoids are found in cacao (Theobroma cacao L.) pod husk extract s from three groups of compounds, i.e. catechin 37%, anthocyanin 4% and tannin 58% and quercetin. In addition, the cacao pod husk is also rich of methylxanthine, caffeine, theobromine, theophylline and minerals such us magnesium, copper, potassium and iron. The potential of cacao pod husk may suppress inflammatory processes by activating the interleukin-1β (IL-1β) and interleukin-2 (IL-2) cytokines as proinflammatory mediators which activate the leukocytes to perform phagocytosis against tissue debris [12], that make the inflammation process completes quickly into the next wound healing phase i.e. the proliferation phase. In addition, the potential of cacaopod huskmay also increases TGF-β as a growth factor which plays an important role in increasing the number of cells thus the wound healing process occurs properly.

Based on the results of research, the average number of fibroblasts cells in the decapitated group on the 3rd day increased. The average number of fibroblast cells between the control group and the treatment group was significantly different (p<0.005). This occurs because the 3rd day is the end of the inflammatory phase and the beginning of the fibroblastic phase. In the inflammatory phase, there is infiltration of acute inflammatory cells to the injured area that next perform bacterial phagocytosis, it is likely to be affected by the activity of the substances contained in the cacao pod husk. Flavonoids, especially proanthocyanidin (tannins) contained in cacao pod husk extract have the ability as an antibacterial agent that works by coagulating or agglomerating bacterial protoplasm [13].

In the 7th group observation, the average number of fibroblast cells between the control group and the treatment group was significantly different (p<0.005). The results obtained are in accordance with the hypothesis that the number of fibroblast cells will begin to rise on the 7th day up to the 10th day. The increase of fibroblast cells is possibly caused by the substances contained in the cacao pod husk. Flavonoids play a role in wound healing, especially in the increase of fibroblast cells. Flavonoids, especially catechins and anthocyanidins, act as anti-inflammatory agents by inhibiting the release of arachidonic acid and release of the lysosomal enzyme from the membrane by blocking the path of cyclooxygenase and lipoxygenase in the inflammation. The suppression of prostaglandins and leukotrin as an inflammatory mediator may lead to less pain in swelling, reduced vasodilation of blood vessels and local blood flow, resulting in migration of inflammatory cells in the inflammatory area reduced. Furthermore, the inflammatory reaction will take place shorter and can immediately enter the next healing stage.

The 10th group observation, the average number of fibroblast cells between the control group and the treatment group was significantly different (p<0.005). Flavonoids contained in the cacao pod husk may lead to decreased levels of proinflammatory cytokines i.e. through inhibition to Nuclear Factor Kappa B (NF-κB). NF-κB becomes active because of the stimulus of ROS agents that cause DNA damage and physical stress. NF-κB functions in controlling the expression of genes encoding proinflammatory cytokines, prostaglandins, nitric oxide (NO) and cell adhesion. Inhibition to transcription factor NF-κB will suppress the production of proinflammatory cytokines; thus the inflammatory reaction will be shorter and the proliferative ability of the growth factors is not inhibited [14].

In addition, cacao pod husk is also rich in methylxanthine, caffeine, theobromine and theophylline, which act as antioxidants by capturing reactive oxygen species (ROS) from granulocytes and activated lymphocytes. The proliferation process of fibroblast cells will stop if the tissue has undergone a perfect repair process. Fibroblasts have a very big role on the tissue repair process that is responsible for the preparation of protein structures product that will be used during the process of tissue reconstruction [15].

CONCLUSION

It is concluded that the addition of cacao pod husk extract has the potential to increase the number of fibroblast cells. In addition, it is suggested to perform further research on the active substance of cacao (Theobroma cacao L.) pod husk extract, as herbal medicine, in increasing number of fibroblast cells which may accelerate the process of wound healing post-tooth extraction.

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AUTHORS CONTRIBUTIONS

All the authors have made substantial contributions to the work reported in the manuscript.

CONFLICT OF INTERESTS

There are no conflict of interest in this study

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