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Research Article

The Effect of Chitosan-Gelfoam Cacao Pod Husk on Wound Epithelial Thickness in the Post-Extraction Tooth with Anticoagulant Therapy

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Abstract

Tooth extraction is removing a tooth from its socket when it cannot be restored. Tooth extraction performed in patients on anticoagulant therapy can increase the risk of excessive bleeding. Therefore, a local hemostatic agent is needed to accelerate the hemostasis process and reduce the risk of tooth extraction complications in anticoagulant users. This study aims to determine the effect of chitosan-gelfoam extract of cacao pod husk to increase epithelial thickness in the post-extraction socket of male Wistar rats with anticoagulant therapy. This research used quantitative data collection techniques on 24 male Wistar rats who were given anticoagulant therapy. It was divided into eight groups: negative control group H+3, negative control group H+7, positive control with oral tranexamic acid therapy H+3, positive control with oral tranexamic acid therapy H+7, treatment 1 with 1 ml chitosan-gelfoam cacao pod husk extract therapy H+3, treatment 1 with 1 ml chitosan-gelfoam cacao pod husk H+7, treatment 2 with 10 ml chitosan-gelfoam cacao pod husk H+3, and treatment 2 with 10 ml chitosan-gelfoam cacao pod husk H+7. The test animals were decapitated on the 3rd and 7th day. The histology preparations, observations, and statistical tests were then carried out. The statistical tests showed that chitosan gelfoam extract of cacao pod husk with doses of 1.6% and 15% extract of cacao pod husk was able to replace the effectiveness of tranexamic acid. The 15% dose had better significance than the 1.6% dose. This research is expected to contribute to the farmers' welfare and the cacao industry by converting cacao pod waste into helpful medicine.

Keywords: anticoagulant; cacao pod husk; chitosan-gelfoam; epithelium; tooth extraction

INTRODUCTION

According to a survey from the International Collaborative Partnership for the Study of Atrial Fibrillation (INTERAF), the use of anticoagulants increased until 2016.[1] Anticoagulants can inhibit platelet aggregation and disrupt wound healing. Thus, special treatment is needed for actions that cause bleeding. Heparin is one of the most commonly used anticoagulants. This drug acts as a factor Xa inhibitor to inhibit blood clotting.[2,3] One of the dental procedures that cause bleeding is tooth extraction. Tooth extraction is the act of removing a tooth from its socket if it cannot be restored. The ideal tooth extraction is the extraction of a tooth or tooth root that is intact without causing pain, with minimal trauma to the supporting tissue. The extraction wound will usually heal and not cause complications.[4,5] As a result of tooth extraction, the injured tissue will undergo a healing process called re-epithelialization. Re-epithelialization is an important step in the healing of tooth socket wounds. Re-epithelialization plays a role in restoring tissue when a wound occurs after tooth extraction.[6,7]

Indonesia is a country rich in marine products. Chitosan is material from

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crustacean shells that are easily decomposed, able to suppress microbial growth, and have no toxic properties, so it is very friendly to the environment.[8,9] The content of lysozyme enzymes and amino polysaccharide groups in chitosan has the potential to be antimicrobial and can be used as a natural preservative.[10] Chitosan has antioxidant, anti-inflammatory, anti-allergic, and antitumor properties and can be a clotting agent.[11] However, chitosan has not been able to produce antioxidants optimally. This deficiency can be overcome by adding materials and modification of chitosan. The proper modification will produce good antioxidant and antibacterial compounds compared to other compounds using only chitosan.[12] The combination of chitosan and other bioactive molecules improves mechanical properties, protein absorption, and biomineralization.[13]

On the other hand, Indonesia is the third country in the world, with a total production of 593,832 tons.[14,15] Cacao pods are the most crucial part, which is often wasted due to the high production of cacao beans. It can also cause environmental pollution.[16,17] Cacao pods contain alkaloids, flavonoids, phenolics, tannins, and saponins which help promote collagen growth and the formation of the new epithelium (re-epithelialization), which shortens wound healing.[18,19] The absence of commercial herbal gel foam products has encouraged researchers to innovate a combination of chitosan and the active ingredient of cacao peel extract to support the effectiveness of gelfoam. Therefore, the researchers innovated chitosan-gelfoam cacao pod husk to speed up wound healing after tooth extraction, as seen from the average thickness of the tooth socket epithelium of Wistar rats given anticoagulant therapy.

MATERIALS AND METHODS

The main ingredients used were 200 grams of cacao pod shell powder from UPT Materia Medica Batu, Indonesia, and chitosan (Black Tiger shrimp shell) with a deacetylation rate of 70% - 87.5% from Lampung, Indonesia. The other materials used were bovine gelatin, 70% ethanol, 70% acetone, 1% acetic acid, rotary evaporator, water bath, distilled water, magnetic stirrer, sonicator, heparin, ketamine, tranexamic acid, normal saline, syringe, alcohol, digital scale, Erlenmeyer, glass stirrer, light microscope, 10 % formaldehyde buffer solution, freeze dryer, Petri dish, object-glass, deck glass, microtome, micropipette, and white rat Wistar strain male (200-250 gr). This study used an experimental design in an in vivo laboratory. The research was conducted at the Biochemistry Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Laboratory of Oral Biology, Faculty of Dentistry, Universitas Brawijaya, Animal house, Faculty of Dentistry, Universitas Brawijaya, UPT Materia Medica Batu, Laboratory of Kessima Medika, Laboratory of Anatomical Pathology, Faculty of Medicine, Universitas Brawijaya, and the Institute of Bioscience, Universitas Brawijaya. The Research Ethics Commission has approved the procedure and sampling of this research of Universitas Brawijaya through the Certificate of Eligibility for Research Ethics (ethical clearance) No.085-KEP-UB-2020 dated August 31, 2020.

Animal Experiment Treatments

The test animal subjects were divided into eight groups: negative control group H+3, negative control group H+7, positive control with oral tranexamic acid therapy H+3, positive control with oral tranexamic acid therapy H+7, treatment 1 with 1 ml chitosan-gelfoam cacao pod husk extract therapy H+3, treatment 1 with 1 ml chitosan-gelfoam cacao pod husk H+7, treatment 2 with 10 ml chitosan-gelfoam cacao pod husk H+3, and treatment 2 with 10 ml chitosan-gelfoam cacao pod husk H+7. Each group consisted of 3 white male rats of the Wistar strain aged 4-5 months, weighing 200-250 grams. In total, 24 rats

were acclimatized for 14 days in the Animal House Laboratory, Faculty of Dentistry, Universitas Brawijaya. The group of rats was divided into four, namely the negative control group (K-), the positive control group (K+) given oral tranexamic acid 4.5 g/200 g BW rats, treatment group 1 (P1) given chitosan-gel foam with cacao pod husk extract 1,6 % and treatment group 2 (P2) were given 15% chitosan-gel foam with cacao pod husk extract. All groups were injected with heparin 0.09 ml/200 g BW, extracted from the left mandibular incisor, and decapitated on the 3 and 7 days.

The Procedure for Extracting Cacao Pod Husk

The first step in making cacao pod husk extract was cleaning, cutting, and sun drying the cacao pod husks. The sun-dried cacao pod husk was then blended into a powder. Seven hundred gram cacao pod husk powder was mixed with 70% ethanol 1.400 ml, and the mixture was stirred using a shaker. After that, the cacao powder was filtered using a filter cloth. The liquid filtrate was put in a rotary evaporator. The water bath was set at 50°C, a boiling point of 30°C, and a pressure of 102 mbar until all the ethanol evaporated. The cacao pod husk extract was packaged in a bottle when it finished.

The Procedure of Chitosan-Gel Foam

One gram of chitosan and 1% (v / v) acetic acid solution of 1 ml in 100 ml of distilled water, gelatin solution (collagen bovine 10 g) in 100 ml of water, and 1 gram of chitosan were prepared. The first step was to mix 1 gram of chitosan and acetic acid solution using a magnetic stirrer at 60° C at 500 rpm for 15 minutes. The gelatin (bovine collagen 10 g) was then dissolved in 100 ml of water, and the solution was stirred using a magnetic stirrer until homogeneous. Next, the gelatin solution was mixed with chitosan and acetic acid in a magnetic stirrer until an opaque solution was formed with a ratio of 3: 1 (chitosan: gelatin). The mixture was divided into two parts with 55 ml portions each. The first part used 1 ml of cacao pod husk extract, and the second used 10 ml of cacao pod husk extract. They were then stirred using a magnetic stirrer at room temperature at 1000 rpm until homogeneous. The final pH of each sample was measured until it was 4.5, and the solution was put in each separate petri dish. The Lyophilization process was carried out until the specimens were solid and formed a sponge slice in sterile conditions according to the size of the rat tooth socket using a laminar airflow. The sliced sponge was covered with plastic wrap and stored in the freezer.

Preparation of Tranexamic Acid and CMC Na

Twenty five g of CMC Na with 50 ml of aqua bidest was mixed. Next, the CMC Na solution was added to the negative control group (K-), about 2.5 ml each. After that, the 31.5 g of tranexamic acid powder was mixed into a 20 ml container, 20 ml of 0.5% CMC Na solution was added and given to the positive control group (K+). The solution was mixed using the vortex mixer and given to rats in 2.5 ml each.

Heparin Induction and Surgical Animal Testing

All treatment groups were injected using heparin at a dose of 0.09 ml/20 g BW rats subcutaneously, 4 hours before extraction. The test animals were divided into eight groups based on the day of observation, namely the third and seventh days, with three rats per subgroup. Animals were anesthetized with 0.25 mg/200 g BW of ketamine in mice. The root of the lower-left first incisor was separated from the gingiva using a lecron, and the tooth was extracted using a needle holder. The K-group was given 0.5% CMC Na (2.5 ml per probe), and the K+ group was given oral tranexamic acid dissolved in 4.5 mg/200 g BW of CMC Na solution. The P1 group was given a dose of 1 ml of chitosan-gelfoam cacao husk extract, and the P2 group was given a dose of 10 ml of chitosan-gelfoam

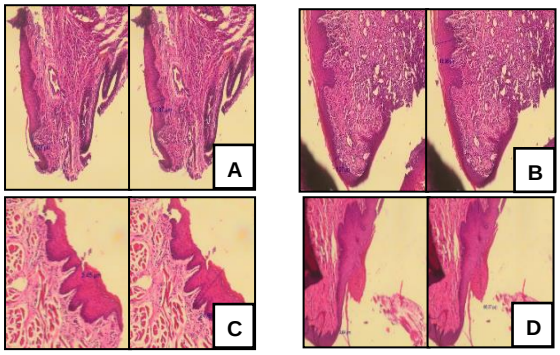
cacao husk extract. The wound was sutured with a braided silk surgical suture. Mice were given analgesics and antibiotics after extraction.

Histological Slide Preparation

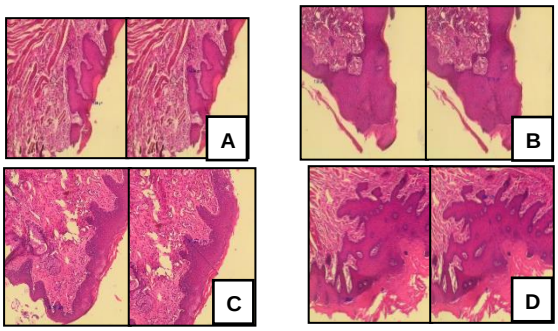
The tested animals were decapitated on the 3rd and 7th days. Decapitation was performed by placing a 10% formalin buffer solution into a 20 ml small container using a 50 ml pipette, naming each container, and closing it. Rats were injected with ketamine until they passed out, then the rat jaws were cut. The rat bodies were later buried appropriately. Tissue fixation was conducted using 10% formalin buffer solution for 24 hours, decalcification was carried out with 10% solution with rapid decal, and a paraffin block was made. The paraffin blocks were cut with a thickness of ± 4-5 µm with a microtome and stained with Hematoxylin Eosin. Preparation of preparations was intended for histological examination of the thickness of the epithelium in the socket with a magnification of 100x.

RESULT

The histopathological analysis of test animals was intended to analyze the epithelial thickness in sockets. Epithelial thickness measurement results were obtained by finding the average width of the thickest and thinnest epithelium in 3 different fields of view using LC Micro software with 100x magnification. Epithelial thickness data on days 3 and 7 from each group were tabulated.



Picture 1. Histopathological pictures of epithelial thickness on the 3rd day of post-extraction. (a) Negative control group, (b) Positive control group (oral tranexamic acid), (c) First treatment group (chitosan-gel foam with 1.6% cacao pod husk extract), (d) Second treatment group (chitosan-gel foam with 15% cacao pod husk extract).



Picture 2. Histopathological pictures of epithelial thickness on the 7th day of post-extraction. (a) Negative control group, (b) Positive control group (oral tranexamic acid), (c) First treatment group (chitosan-gel foam with 1.6% cacao pod husk extract), (d) Second treatment group (chitosan-gel foam with 15% cacao pod husk extract).

Table 1. Average epithelial thickness (µm) on the 3rd and 7th day of the tooth socket

Mean	Day-3	Day-7
K-	7,21 µm	21,23 µm
K+	10,36 µm	31,60 µm
P 1	14,61 µm	35,52 µm
P 2	24,52 µm	41,52 µm

Description:
K-: Negative control group
K+: Positive control group
P1: First treatment group (chitosan-gel foam with 1.6% cacao pod husk extract)
P2: Second treatment group (chitosan-gel foam with 15% cacao pod husk extract).

The results of epithelial thickness measurements in the control and treatment groups were analyzed using the Kruskal-Wallis test and continued with the Mann-Whitney analysis (95% confidence level) using the Statistical Package for the Social Sciences (SPPS) version 25.0 software. Based on the Kruskal-Wallis test, it can be seen that the epithelial thickness data on the 3rd and 7th day had a p-value ≤ 0.05, indicating a significant difference in mean epithelial thickness between all groups.

Table 2. *Mann Whitney* Test

Group		Sig.	Conclusion
K-	K+	0.050	Significant
	P1	0.050	Significant
	P2	0.050	Significant
K+	P1	0.050	Significant
	P2	0.050	Significant
P1	P2	0.050	Significant

Furthermore, the Mann-Whitney statistical test was performed to determine the differences in each group in detail. The results of the discussion of the comparison between groups showed the comparison between the Negative Control Group (K-) with the Positive Control Group (K+), Treatment Group 1 (P1), and Treatment Group 2 (P2) at H+3 obtained 0.050 as a p-value. This value is less than equal to 5% ($0.05 \leq 0.05$), indicating a significant difference. The comparison between the Positive Control Group (K+), with Treatment Group 1 (P1) and Treatment Group 2 (P2) at H+3 obtained 0.050 as a p-value. This value is less than equal to 5% ($0.05 \leq 0.05$), indicating a significant difference. The comparison between Treatment Group 1 (P1) and Treatment Group 2 (P2) at H+3 obtained 0.050 as a p-value. This value is less than equal to 5% ($0.05 \leq 0.05$), indicating a significant difference. The comparison between the Negative Control Group (K-) with the Positive Control Group (K+), Treatment Group 1 (P1), and Treatment Group 2 (P2) at H+7 obtained 0.050 as a p-value. This value is less than equal to 5% ($0.05 \leq 0.05$), indicating a significant difference. The comparison between the Positive Control Group (K+), with Treatment Group 1 (P1) and Treatment Group 2 (P2) at H+7 obtained 0.050 as a p-value. This value is less than equal to 5% ($0.05 \leq 0.05$), indicating a significant difference. The comparison between Treatment Group 1 (P1) and Treatment Group 2 (P2) H+7 obtained 0,050 as a p-value. This value is less than equal to 5% ($0.05 \leq 0.05$), indicating a significant difference.

DISCUSSION

Tooth extraction is an action from the socket that can cause trauma to the supporting tissue. Injured tissue will undergo a healing process or re-epithelialization. Re-epithelialization is a step that is one of the main parameters in the wound healing process, where the faster the re-epithelialization is, the faster the wound healing will be. Re-epithelialization plays a role in restoring tissue integrity when a wound occurs after tooth extraction.[7,20] The occurrence of thick epithelium can be proven by measuring the width of the epithelial gap formed. The epithelialization process strongly influences wound healing; the faster the wound closes, the faster the wound healing will be.[21] The results of the observation were the negative group H+3; the control group was negative H+7, the positive control group was treated with oral tranexamic acid H+3, the positive control group was treated with oral tranexamic acid H+7, treatment 1 was treated with 1 ml of chitosan-gelfoam cacao pod peel extract therapy H+3, treatment 1 was treated with 1 ml chitosan-gelfoam cacao pods H+7, treatment 2 was treated with 10 ml chitosan-gelfoam cacao pods H+3, and treatment 2 was treated with 10 ml chitosan-gelfoam cacao pods H+7. The results of the cacao pod peel significantly helped the wound healing process after tooth extraction in anticoagulant users. Treatment 2 with 10 ml of chitosan-gelfoam cacao pod skin H+7 or a concentration of 15% was proved to be the most effective for increasing the epithelium. Previous research by Kurniawati discussed that cacao bean extract gel effectively accelerated the wound healing process.[22] In this study, the researchers innovated gel foam combined with cacao pod peel extract, which also contained compounds such as alkaloids, flavonoids, phenolics, tannins, and saponins that helped increase collagen growth and new epithelial formation that have anti-inflammatory effects. The anti-

inflammatory properties found in cacao pods could reduce the expression of MCP-1, which produced inflammatory cytokines so that the inflammatory process became shorter and the proliferative ability of TGF-β was not inhibited. The TGF-β and KGF which are growth factors, play an essential role in the process of keratinocytes in epithelialization and are evidenced by an increase in epithelial thickness. Chitosan, which has biocompatible, biodegradable, non-toxic, safe, and antitumor properties, can be used in drug delivery systems and is suitable for use as a hemostatic agent. Therefore, chitosan-gelatin and cacao pod skin formed on hemostatic agents provide the maximum effect in wound healing after tooth extraction, seen from the increased epithelial thickness, especially in the Wistar rat model that was injected with heparin and had impaired wound healing. Furthermore, the limitations of the research carried out in this study include the combination and concentration of herbal ingredients used for further exploration so that it is possible to get the best concentration. The researcher recommends that further research can be carried out related to stabilization, dispersibility, and homogeneity of the chitosan gelfoam cacao shell to identify its use limits.

CONCLUSION

In this research, chitosan-gel foam with cacao pod husk extract effectively accelerated wound healing by increasing the epithelial thickness of the tooth socket at post-extraction in anticoagulant users. Based on the Kruskal-Wallis statistical test result followed by the Mann-Whitney statistical test, cacao pod husk extract doses of 1.6% and 15% were proved to be more effective than tranexamic acid with the most effective concentration being 15%. Further research is needed to determine the effectiveness of chitosan-gel foam cacao pod husk with different cacao pod husk extract doses.

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