Oral mucoadhesive patch of green betle leaf, areca nut, and gambier can reduce the size of traumatic ulcer lesion

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ABSTRACT

Background: *Although topical corticosteroids and antibiotics are frequently prescribed to treat traumatic ulcers, both medications have disadvantages. In addition, topical application of the drug is ineffective because it can be washed off by saliva. In order to speed up the healing of traumatic ulcers, it is believed that the oral mucoadhesive patches will adhere to the ulcer for a longer amount of time.*
Method: *This study was a pure laboratory experiment with a post-test only randomized control group design. The study samples were 32 white rats (Rattus norvegicus) of the Wistar strain with traumatic ulcers on the buccal mucosa. The sample was divided into 2 groups: (1) the control group, which was only given analgesics (5% EMLA); and (2) the treatment group, which was given analgesics (5% EMLA) and oral mucoadhesive patches (a combination of ethanol extracts of green betel leaf, areca nut, and gambier). Each group received treatment twice a day for 10 days. Then the diameter of the traumatic ulcer lesion was measured with a digital caliper on days 1–10 from the yellowish-white part. The data obtained were analyzed using an independent t-test.*
Results*: There was a significant difference in the diameter reduction of traumatic ulcer lesions between the control group and the treatment group ($p<0.05$). Healing of traumatic ulcers in the treatment group was faster than in the control group.*
Conclusion: *An oral mucoadhesive patch combination of ethanol extracts of green betel leaf, areca nut, and gambier is effective in reducing the size of traumatic ulcer lesions.*

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INTRODUCTION

Traumatic ulcer is one of the disorders of the oral cavity that often occurs in the community and is characterized by ulceration due to loss of the epithelial layer that extends to the basement membrane.[1] A traumatic ulcer caused by several factors, including physical, thermal, and chemical trauma.[2] Traumatic ulcer are erythematous, with raised edges and a necrotic center that is yellowish white, and usually heal in about 10 days after removing the factor that caused the wound. This disorder can be diagnosed in comparison with other disorders based on the size of the lesion, location, number, onset, age of the patient, the patient's congenital disease, and the development of the wound.[3] When a wound occurs, the body has a physiological response in the form of a wound healing process. Like the oral mucosal tissue, physiologically it goes through basic healing processes consisting of hemostasis, inflammation, proliferation (called granulation tissue and contraction), and remodeling or maturation. The healing process of a traumatic ulcer wound may be seen macroscopically or clinically from the dimensions of the wound, including its length, depth, and diameter.[4]

Some medications that are often used to repair damaged tissue in the healing process of traumatic ulcers and preventing infection are topical corticosteroids and antibiotics,[5] but the use of these two drugs has drawbacks. The use of corticosteroids can lead to the growth of Candida albicans in the oral cavity,[6] while the excessive use of antibiotics causes microorganisms to become resistant to antibiotics so that antibiotics do not work properly to cure infections.[7]

Recently, the use of mucoadhesive polymers in drug delivery systems has developed. Mucoadhesive drugs in patch form provide greater flexibility and convenience than other dosage forms. In addition, oral mucoadhesive patches can avoid the ineffectiveness of drugs applied in gel form because they are rubbed off by saliva. The application of drugs using oral mucoadhesive patches is expected to allow the drug to stick to the ulcer longer so that it is more effective in accelerating the healing of traumatic ulcers.[8]

Indonesia is a country with abundant natural wealth, including herbal plants that have been used for generations to treat various diseases.[9] The use of natural ingredients as a treatment is in demand because it does not cause any side effects.[10] Previous studies have reported that natural ingredients such as betel leaf (Piper betle L.), areca nut (Areca catechu), and gambier (Uncaria gambir) have broad-spectrum therapeutic properties. Some of the resulting therapeutic properties include anti-plaque, anti-diabetic, anti-inflammatory, analgesic, wound healing, and antibacterial. In general, plants that are rich in phenolics, such as those contained in these three ingredients, have high antioxidant properties, so that they provide a significant therapeutic effect.[11,12] In addition, the use of a combination of betel leaf ethanol extract, areca nut, and gambier in various preparations has a synergistic effect as an antibacterial.[11,12,13,14]

This study aims to prove the effectiveness of an oral mucoadhesive patch combination of ethanol extracts of green betel leaf (Piper bitle L), areca nut (Areca catechu), and gambier (Uncaria gambir) in reducing the size of traumatic ulcer lesions in white rats (Rattus norvegicus) of the Wistar strain.

RESEARCH METHOD

The research conducted was a pure laboratory experiment with a post-test only randomized control group design. The research has an ethical eligibility number of 751/VI/HREC/2022 from the Health Research Ethics Commission

(KEPK), RSUD Dr. Moewardi. The study began with plant determination, which was carried out at the Laboratory of Plant Systematic Morphology, Universitas Setia Budi (No.13/Det.Lab/VI/2022, No.14/Det.Lab/VI/2022, and No.15/Det.Lab/VI/2022), then proceeded with the manufacture of extracts of green betel leaves, areca nut, and gambier. The extraction of the three ingredients was carried out by maceration using 70% ethanol as solvent to obtain ethanol extracts of betel leaf, areca nut, and gambier with a concentration of 0.5% each.

The extracts of the three materials that have been obtained are then made into oral mucoadhesive patches using the solvent casting technique with the addition of HPMC, Na-CMC, propylene glycol, Tween 80, and distilled water with a predetermined formulation. Furthermore, the oral mucoadhesive patch was subjected to physical tests to determine the physical properties of the formulations that had been made. The physical tests included a thickness test using a digital caliper, a weight uniformity test with an analytical balance, a pH test with a pH meter, and a folding endurance test.

Figure 1. Oral Mucoadhesive Patch Preparations Combination of Ethanol Extract of Green Betel Leaf, Areca Nut, and Gambier

The oral mucoadhesive patch measuring 0.5 x 0.5 cm (Figure 1) was then tested on test animals consisting of white male rats of the Wistar strain that had been acclimatized for 1 week. Prior to the application of oral mucoadhesive patches, the test animals were first subjected to the formation of traumatic ulcer lesions on the buccal mucosa with a punch biopsy of 3 mm diameter and 1 mm depth. The trick is that all test animals were anesthetized using ether, and then the analgesic lidocaine prilocaine (EMLA 5%) cream was applied to the buccal mucosa. Traumatic ulcer lesions were made using a punch biopsy by stretching the mucosa perpendicular to Langer's line, then inserting the punch biopsy vertically in a circular motion.

The treatment was given twice a day, in the morning and evening, by applying analgesics (EMLA 5%) to the control group, while the treatment group received analgesics (EMLA 5%) and oral mucoadhesive patches made of a combination of ethanol extracts of green betel leaf, areca nut, and gambier. Checking the condition of the patches was carried out periodically to ensure that they were still attached to the buccal mucosa of the rats. Each group consisted of 16 test subjects. From the first to the tenth day, the diameter of the traumatic ulcer lesion was measured with a digital caliper from the yellowish-white part (Figure 2). The data that has been obtained was analyzed statistically using an independent t-test.

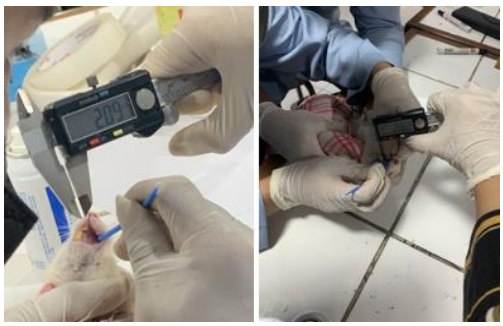

Figure 2. Measurement of Traumatic Ulcer Lesion in Test Animals

RESULTS

The results of the physical evaluation of the oral mucoadhesive patch combination of ethanol extracts of betel leaf, areca nut, and gambier are shown in Table 1. The thickness test showed an average of 0.21 mm, which means that the oral mucoadhesive patch met the requirements because the thickness was less than 0.25 mm. The oral mucoadhesive patch weight uniformity test yielded 8.62 mg with a standard deviation of 0.1, which means that the weight was fairly uniform. The results of the pH 7.0 test showed that the oral mucoadhesive patch was safe to use and did not irritate the oral mucosa.[15] Based on the results of the folding endurance test, it is known that the mucoadhesive patch has quite good strength because the patch remains unbroken or there is no breaking after >250 folds. This shows the durability of the oral mucoadhesive patch is good and has flexible properties.[16]

Table 1. Physical Evaluation Test Results for Oral Mucoadhesive Patches Combination of Ethanol Extracts of Green Betel Leaf, Areca Nut, and Gambier

Physical Evaluation of Oral Mucoadhesive Patches	Results
Thickness measurement (mm)	0.21
Weight uniformity test (mg)	8.62 ± 0.1
pH test	7
Folding endurance test	>250

The oral mucoadhesive patch combination of ethanol extract of green betel leaf, areca nut, and gambier was then tested on white rats of the Wistar strain to determine its effect on the size of traumatic ulcer lesions on the buccal mucosa. The two study groups, namely the control group and the treatment group, showed an average decrease in the diameter of the traumatic ulcer lesions of white rats (Rattus norvegicus) of the Wistar strain from day to day, which were observed on days 1 to 10 as shown in Table 2 and Figure 3.

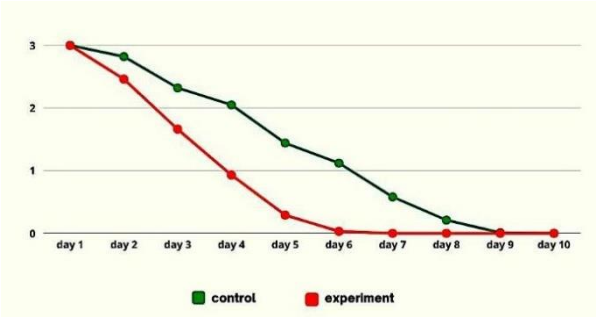

Figure 3. Chart of the Mean Decreased Diameter of Traumatic Ulcer Lesion in White Rats of the Wistar Strain

Table 2 shows that the healing of traumatic ulcers in the Wistar strain white rats in the control group occurred on the 9th day, while in the treatment group it occurred on the 6th day. The data was then tested for normality using the Shapiro-Wilk test. The Shapiro-Wilk test results for the control group had a significance value greater than 0.05 ($p>0.05$), so the data were normally distributed, but for the treatment group, it had a significance value less than 0.05 ($p<0.05$) which means that the data are not normally distributed. Data transformation was performed to normalize the distribution of treatment group data using the log function. Based on the results of the normality test with Shapiro-Wilk, it was found that both data groups had a significant value greater than 0.05 ($p>0.05$), which means that all data were normally distributed, so that an independent parametric t-test could be performed. The results of the t-test are presented in Table 3.

The summary of the t-test in Table 3 obtained a significance value of 0.012, which is less than 0.05 ($p<0.05$), which means that there was a significant difference in the reduction in the size of the lesion diameter from the first to the tenth day between the control group of Wistar strain white rats that only applied analgesics and the treatment group of Wistar strain white rats that were given analgesics and mucoadhesive patches a combination of ethanol extract of green betel leaf, areca nut, and gambier.

Table 2. Mean Diameter of Traumatic Ulcer Lesion in Wistar White Rats

Sample groups	Mean diameter of the lesion (mm)									
	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6	Day-7	Day-8	Day-9	Day-10
Control	3	2.82	2.32	2.05	1.44	1.12	0.58	0.21	0.01	0
Treatment	3	2.46	1.66	0.93	0.29	0.04	0	0	0	0

Table 3. Summary of Independent T-Test

Sample Groups		*t-test* Significance Value
Control	Analgesic (EMLA 5%)	0.012
Treatment	Analgesic (EMLA 5%) and Oral Mucoadhesive Patches (Combination of Ethanol Extracts of Green Betel Leaf, Areca Nut, and Gambier)	

DISCUSSION

Wound healing is a process to repair the skin and soft tissue after a wound has formed. The wound healing process consists of several phases, namely the inflammatory phase, the proliferative phase, and the remodeling phase.[17] The inflammatory phase is divided into two, namely the early and late inflammatory phases. This phase is characterized by macrophages that secrete growth factors such as TGF-β, TGF-α, FGF, and VEGF, which play a role in initiating the next phase, namely the proliferative phase. The proliferative phase plays a full role in tissue regeneration. This process of new tissue formation occurs between the second and twentieth days after the wound is formed. This phase involves migration and proliferation of epithelial cells; myofibroblasts are activated; and capillary proliferation becomes new tissue. Epithelial closure is a phase that involves the migration of keratinocyte cells over the wound tissue. The final phase of wound healing is the remodeling phase. In this phase, fibroblasts differentiate into myofibroblasts.[18] This phase also includes the downregulation of all biological responses that were activated following the wound.[19]

The treatment group in this study showed faster healing of traumatic ulcers than the control group. The reduction in the diameter of traumatic ulcer lesions in white rats of the Wistar strain (Rattus norvegicus), which was faster in the treatment group as seen in Figure 3, shows the healing effect of the oral mucoadhesive patch of a combination of ethanol extract of green betel leaf, areca nut, and gambier on traumatic ulcer lesions.

The healing activity of traumatic ulcer lesions in the treatment group given the mucoadhesive patch combined with the ethanol extract of green betel leaf, areca nut, and gambier can be influenced by the immunity and nutritional intake of the test animals,[20] as well as the action of the active substance contained in the mucoadhesive patch combined with the ethanol extract of green betel leaf, areca nut, and gambier. Flavonoids contained in green betel leaves have the ability to act as antioxidants, which work by increasing vascularization and inhibiting lipid peroxidation so as to prevent tissue damage. This causes an increase in the formation of collagen fiber strength, which affects cell proliferation, increases epithelialization, and increases the formation of

melanin, melanocytes, and fibrous tissue in the process of wound healing.[21,22]

In the process of wound healing, the content of flavonoids also plays a role by inhibiting cyclooxygenase and lipooxygenase, so that the number of inflammatory cells that migrate to the wound tissue will be limited and will cause a short inflammatory reaction. Saponins in betel leaves also play an important role in wound healing by stimulating the synthesis of fibronectin by fibroblasts and changing the expression of TGF-β receptors.[5] Fibronectin is present in the early phase of wound healing and induces fibroblast migration. Fibroblasts will produce collagen, so the more fibroblasts that migrate to the wound area, the greater the increase in collagen synthesis. This causes the collagen in the extracellular matrix to thicken, and the wound will shrink, thus accelerating wound healing.[6,23]

Arecoline is the main alkaloid in areca seed extract, which acts as an analgesic and anti-inflammatory with a molecular mechanism that effectively reduces the production of pro-inflammatory cytokines IL-6, IL-1, and COX-2 expression and simultaneously increases the amount of IL-4 anti-inflammatory cytokines.[24] Tannin contained in areca nut seeds in the wound healing process acts as an anti-oxidant as well as an astringent, namely accelerating wound healing and inflammation of mucous membranes. Tannins also have the ability to induce TGF-β, which will trigger fibroblast migration and proliferation, resulting in a new tissue regeneration process.[9,25]

The catechins contained in gambier can work to reduce the expression of IL-6 and IL-8 so that they can be used to overcome inflammation and improve the wound healing process by accelerating the formation of fibroblasts, accelerating migration, and stimulating the deposition of tenascin, fibronectin, and collagen I during wound healing.[26] Catechins can also keep wounds sterile and stimulate the wound healing process in ulcer lesions.[12]

Various active substances contained in extracts of betel leaf, areca nut, and gambier caused faster healing of traumatic ulcer lesions in the treatment group of white Wistar rats, which was indicated by a significant reduction in the diameter of the traumatic ulcer. This is in accordance with previous studies, which said that betel leaf (Piper betle L.), areca nut (Areca catechu), and gambier (Uncaria gambir) have broad-spectrum therapeutic properties because these three plants are rich in phenolics so that they provide significant therapeutic effects.[11]

Oral mucoadhesive patches containing natural ingredients are effective in healing traumatic ulcers. The natural ingredients used are a combination of the ethanol extract of green betel leaves (Piper bitle Linn), areca nut (Areca catechu), and gambier (Uncaria gambir). The material is formulated in the form of an oral mucoadhesive patch because it is considered to have advantages, namely being able to stick to traumatic ulcers for a long time, being flexible so that it is comfortable when applied, and besides that it can avoid the ineffectiveness of the drug due to being rubbed off by saliva.[27,28]

CONCLUSION

Oral mucoadhesive patches containing ethanol extracts of green betel leaf, areca nut, and gambier are effective in reducing the diameter of traumatic ulcer lesions in white rats (Rattus norvegicus) of the Wistar strain.

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