ORIGINAL ARTICLE

Anti-bacterial Effect of Chitosan Mucoadhesive Patch Combined With Rambutan Peel Extract (*Nephelium Lappaceum*) on Decreasing the Number of Porphyromonas Gingivalis

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ABSTRACT

Introduction: The increase in gingivitis cases based on RISKESDAS data in 2018 is an urgency that requires alternatives to prevent the recurrence of the increase in gingivitis cases in Indonesia. Gingivitis can continue to spread to hard tissue lesions and develop into periodontitis, leading to tooth loss or tooth loss if not treated right away. Chitosan Patch as a treatment for gingivitis can active in a focuse conducting in a focused manner on the target site because it does not go through first-pass metabolism in the liver. The rind of rambutan fruit can be anti-inflammatory and antibacterial with a high content of polyphenols and flavonoids. **Methods:** The study was divided into five groups, namely two control group and three treatment groups, each treated using a chitosan mucoadhesive patch combined with rambutan peel extract with concentrations of 5%, 10%, and 15%. On the sixth day after patching, *Porphyromonas gingivalis* bacteria were cultured on the rat gingiva, and bacterial colonies were counted using a colony counter and continued with data analysis. **Results:** The results showed that the mucoadhesive patch of chitosan combined with rambutan peel extract could significantly reduce the number of *Porphyromonas gingivalis* bacterial colonies in all groups (P>0,05). **Conclusion:** Chitosan mucoadhesive patch combination of rambutan peel extract can be an alternative therapy as an antibacterial against gingivitis.

Keywords: Mucoadhesive patch; Chitosan; Rambutan peel extract; Gingivitis; *Porphyromonas gingivalis*

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INTRODUCTION

Based on data from the 2018 RISKESDAS related to periodontal disease, there was an extremely increase in gingivitis cases from 23.3% in 2007 to 57.6% in 2018. Gingivitis has a very high prevalence rate in Indonesia, which is 74%. From this prevalence rate, gingivitis is the second most common periodontal disease in Indonesia.

Gingival inflammation is an inflammatory disease of the gingiva that occurs in the soft tissue areas of the gingival epithelium and connective tissue without swelling of the alveolar bone, periodontal ligament, or cementum, so that adhesion to the teeth is not lost (Attachment loss) (1,2). Gingivitis is commonly found in communities with the clinical features of pain, discomfort, swelling, redder than regular gum color changes, and bleeding when touching or applying pressure to inflamed areas (3). Gingivitis can continue to spread to hard tissue lesions and develop into periodontitis, leading to tooth loss or tooth loss if not treated right away. Based on research by Trombelli, et al., 2018, the most significant prevalence as the etiology of gingivitis is a bacterial invasion to the periodontal tissue. The dominant agent as the most significant etiological factor in the pathogenesis of gingivitis and other periodontal diseases is Porphyromonas gingvalis (4).

The primary treatment commonly used in cases of gingivitis is scaling and planning, an attempt to clean up plaque and tartar where many disease-causing bacteria accumulate. In addition, additional therapy is required to accelerate the healing of inflamed tissues and accelerate the reduction of subchondral and tissue bacterial accumulation. Commonly used adjunctive therapy is an antiseptic with the compound chlorhexidine in mouthwash preparations. Chlorhexidine in mouthwash preparations has

several worrisome side effects, including irritation of the oral mucosa, leaving an uncomfortable burning sensation, causing staining or discoloration of teeth and increasing the formation of subgingival calculus (5,6). In addition, the drug contact time with the inflamed tissue tends to be short (5).

As a treatment for gingivitis, the Chitosan patch has flexibility, for using at long stay time, can close the wound on the mucosa, effectively relieves pain, does not cause irritation, and is comfortable when living (7,8). Chitosan has biocompatibility, biodegradation, and mucosal adhesion properties that allow the patch preparation to adhere to the gingival mucosa and temporarily open the tight junction of epithelial cells, an essential factor critical in drug delivery (9,10). Chitin is a source of chitosan that can be obtained from the shells of blood clams (Anadara granosa) abundant in Indonesia. Blood cockle shells contain 1435% chitin, and then chitin reduction will yield 89.95% total chitosan (8,11). The addition of other materials as active ingredients during patch preparation is necessary to optimize the anti-inflammatory and antimicrobial potential. The natural raw material that can be used as an active ingredient or as a medicine is the peel of rambutan (Nephelium lappaceum L.), which can act as an anti-inflammatory due to ellagic acid, corilagin, and geranine as well as an anti-inflammatory agent. Bacteria due to flavonoids, phenols (polyphenols), and saponins (12).

Therefore, research is needed to determine the reduction in the number of bacterial colonies of *Porphyromonas gingivalis* following a mucosal patch made from a combination of blood cockle (Anadara granosa) shell waste and extracts rambutan (Nephelium lappaceum L.) peel in gingivitis.

MATERIALS AND METHODS

This research has obtained the ethic of Brawijaya University with No. 073-KEP-UB-2021. This study used a proper experimental design research method with a Randomized Group Post-Test Only Design approach. The research was conducted at the Laboratory of Oral Biology, FKG UB, Animal House, FKG UB, Materia Medica Batu, and the Surabaya Industrial Research and Consultation Institute.

The formulation of the mucoadhesive patch preparation using rambutan peel extract used HPMC, PVP, and chitosan (13). Other additional ingredients are tween 80 as an emulsifier/surfactant, glycerin as a plasticizer, and peppermint oil as a flavor enhancer (13). The method used in the manufacture of this formulation is the solvent casting method (13). Chitosan was first dissolved into acetate buffer pH 4, and a thick extract of rambutan rind was dissolved using distilled water. In a different cup, HPMC was dissolved using 96% ethanol to taste, after dissolving added PVP, mixed until homogeneous, then added a solution of rambutan peel extract and chitosan solution stirred until homogeneous and formed a mucilage (13,14). After homogeneous, the mixture was added with glycerin, tween, and peppermint oil (14). Furthermore, all ingredients are mixed until homogeneous. The homogeneous material was poured on a petri dish and dried until a film was formed. After the film is formed, a top layer of the patch is made using HPMC: PVP (3:1), which is also carried out using the solvent casting method. Then leave until dry (13,14).

The sample was divided into five groups, namely the negative control group (K(-)), the group without treatment and control, the group (K(+)), which was only induced by LPS *Porphyromonas gingivalis* without being given the mucoadhesive patch of chitosan combination of rambutan peel extract. The group treatment (KP) was given a mucoadhesive patch of chitosan with a combination of rambutan peel extract at concentrations of 5%, 10%, and 15%. Experimental animals that had been acclimatized for seven days were induced by *Porphyromonas gingivalis* bacteria in the LPS (Lipopolysaccharide) preparation on the gingiva of the mandibular right first incisor as much as 0.01 ml each induction, within two days.

Animals that have experienced inflammation are then treated using a chitosan mucoadhesive patch combined with rambutan peel extract. Patches were applied to the rat's gingiva twice a day for five days. On the sixth day after patching, the gingival crevicular fluid (GCF) was taken using paper point 15 on the gingival sulcus of the rat mandibular first incisor (15). Counting the number of colonies was carried out on bacteria developed in bhi-broth media and then mixed on agar media in a Petri dish using the Pour plate method. After that, incubation was carried out in an anaerobic incubator for 24 hours, and then bacterial colonies were counted using a Colony counter (15).

RESULTS

The results showed that the clinical picture of gingivitis in Wistar rats given a mucoadhesive patch of chitosan combined with rambutan peel extract looked different from the control group (Fig. I). Table I shows the mean and the normality test for the average bacterial colonies.

Table I : Effect of chitosan mucoadhesive patch combined with rambutan peel extract decreases the number of *Porphyromonas gingivalis* bacterial colonies.

Group	n	Mean (SB)	Normality test	Homogenity Test	ANOVA
K(-)	5	21,06 (6,8)	0,802*		
K(+)	5	232,2 (10,3)	0,662*		
KP 1	5	172,0 (33,6)	0,627*	0,077*	0.000**
KP 2	5	140,8 (28,6)	0,424*		
KP 3	5	75,6 (15,0)	0,899*		

(*) p> 0.05; (**) p <0.01

Fig. 1 : The left image of the control group and the right image after being given a chitosan mucoadhesive patch combined with rambutan peel extract.

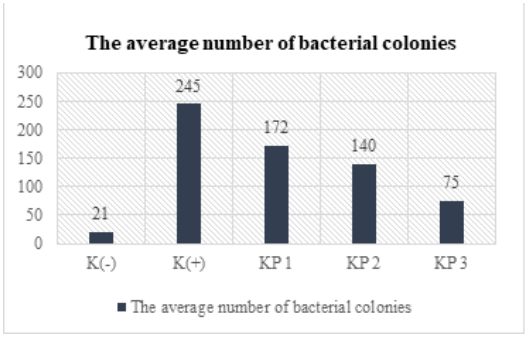

Fig. 2 : Diagram of the average number of bacterial colonies in CFU10^3/ml.

Based on the calculation of the number of bacterial colonies in the gingival sulcus calculated with a colony counter, it was found that white rats with gingivitis who had been given a mucoadhesive patch of chitosan with a combination of rambutan peel extract with a concentration of 15% had the lowest average than the other groups. This can be shown in Fig. 2, which shows the distribution of the average number of bacterial colonies in the control group and the treatment group. From the statistical calculations, it was found that p <0.005, so it can be concluded that there was a difference between the control and treatment groups.

DISCUSSION

Rambutan fruit peel is a natural resource waste easily found in Indonesia; however, it is still rarely used as a medicinal ingredient (16). The content of rambutan peel extracts such as ellagic acid, corilagin, geranine, flavonoids, phenols (polyphenols), and saponins are active compounds that can act as antibacterial and anti-inflammatory (12). This study is in line with research conducted by Karimah et al. (2021), who tested the potential antibacterial activity of rambutan peel extract, and the results showed that rambutan peel extract had moderate to intense antibacterial activity due to the presence of secondary metabolites, namely flavonoids, saponins, and tannins. In addition, it is also supported by research conducted by Rizka and Saptarini (2018) that the metabolite compounds in rambutan peel extract are the basis for their use as functional preparations.

In this study, experimental animals were Wistar rats that *Porphyromonas gingivalis*-LPS had induced. *Porphyromonas gingivalis* is a gram-negative bacterium. The content of flavonoids, phenols, and geraniin in rambutan peel extract is antibacterial because it can damage cell membranes by denaturing and coagulating proteins. Antibacterial compounds in the patch preparation, which have a longer contact time and work locally on the inflamed part, help PMN leukocytes work in phagocytosis and cause a decrease in the number of PMN leukocytes so that bacterial growth is inhibited and there is a decrease in the number of *Porphyromonas gingivalis* bacterial colonies.

CONCLUSION

The results showed a decrease in the number of bacterial colonies of *Porphyromonas gingivalis* after administering a chitosan mucoadhesive patch

combined with rambutan peel extract in gingivitis of Wistar rats (Rattus norvegicus).

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