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Research Report

Chitosan mucoadhesive patch combination of rambutan (*Nephelium lappaceum L)* peel extract decrease leukocyte cell in gingivitis animal model

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ABSTRACT

Background: Gingivitis, or inflammation of the gingiva, is characterized by increased leukocyte cells as a sign of an inflammatory response. A mucoadhesive patch made from chitosan combined with *rambutan* (*Nephelium lappaceum L)* peel extract can reduce inflammation in the gingiva. Chitosan has been proven to inhibit bacterial growth. Meanwhile, rambutan peel extract, as an active ingredient, acts as an anti-inflammatory and anti-bacteria. **Purpose:** This study aimed to examine the impact of administering rambutan (*N. lappaceum L.*) peel extract in chitosan mucoadhesive patches made from blood clam shell waste as a treatment for gingivitis on the total leukocyte cell count in white rats. **Methods:** This research used a true-experimental, post-test-only randomized control group design study, an in vivo study. Wistar rat (*Rattus novergicus*) gingiva was induced with lipopolysaccharide (LPS) *Porphyromonas gingivalis* (Pg) for two days and then treated with a mucoadhesive patch for six consecutive days. Histological preparations were made with hematoxylin eosin (HE) staining using a light microscope to observe the total number of leukocyte cells in Wistar rat (*R. novergicus*) gingival tissue. **Results:** Based on the outcomes of this study, the Kruskal-Wallis statistical test obtained the total number of leukocyte cells, showing a significant decrease ($p < 0.05$) at the active ingredient concentrations of 10% and 15% rambutan (*N. Lappaceum L.*) peel extract in chitosan mucoadhesive patches against the positive control group. **Conclusion:** Rambutan (*N. lappaceum L.*) peel extract in chitosan mucoadhesive patches was able to reduce total leukocyte cell count in gingivitis of Wistar rats (*Rattus novergicus*).

Keywords: Gingivitis; mucoadhesive patches; chitosan; *Nephelium lappaceum L;* medicine

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INTRODUCTION

Riset Kesehatan Dasar (Riskesdas), or Basic Health Research data from 2018, indicate that 74% of Indonesians suffer from gingivitis, an inflammation of the gingiva.[1] Endogenous oral microorganisms that are closely packed together to generate plaque are linked to the development of gingivitis and can lead to the accumulation of potentially inflammatory microbes.[2] The bacteria that cause gingivitis are found in food waste, calculus, plaque, and material alba, all of which are part of the oral hygiene criteria.[3]

A periodontopathogen bacteria called Porphyromonas gingivalis, which is present in plaque, can cause gingival inflammation by producing endotoxins, inducing aberrant antigen-antibody reactions in response to bacterial antigens; and producing enzymes that can hydrolyze the intercellular components of the gingival epithelium and underlying connective tissue.[4] Gingivitis is frequently painless, and those who have it rarely notice it. Gingivitis is characterized by redness, swelling, and bleeding without causing the alveolar bone to be destroyed. The formation of a bacterial biofilm on the teeth or beneath the gingival margin initiates the tissue degradation process.[5]

White blood cells called leucocytes play a key role in the inflammatory response. A rapid and effective defense against potential infectious pathogens is offered by white blood cells. Infections with bacteria and other harmful and infectious microorganisms are characterized by elevated white blood cells. Acute inflammation is caused by white blood cells called monocytes and neutrophils. Additionally, lymphocytes and macrophages are the white blood cells involved in chronic inflammation.[6]

Scaling and root planning, two fundamental treatment methods that are successful in treating plaque and calculus-induced gingivitis, are the primary methods for removing the plaque and calculus that cause gingivitis. Treatment options for gingivitis include mechanical methods like brushing and chemical methods like using mouthwash's antiseptics to get rid of bacteria and tooth decay-causing agents.[7]

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Mouthwash with chlorhexidine is one of the antiseptics used. Gingivitis is prevented, and plaque development is inhibited by 0.2% chlorhexidine. Nevertheless, there are long-term restrictions on this mouthwash. Sloughing of the mucosal membrane and an elevated risk of oral cancer result from using chlorhexidine mouthwash for longer than four months. Chlorhexidine mouthwash should therefore be replaced with a practical, non-irritating, and efficient supplementary treatment for patients with gingivitis.[8]

An approach is to use chitosan as the basic material to create gingival patches. Because they are flexible, efficient, and simple to prepare, mucoadhesive patches have drawn a lot of attention and are the best alternative gingivitis therapies. Chitosan itself is suitable for use as a polymer in gingivitis patch preparations because it can bind to mucin, prevent bacterial growth, and improve patch adhesion to the oral mucosa.[8]

Chitosan was selected as the material for the patch because of its antimicrobial, anti-inflammatory, biocompatible, biodegradable, and bioadhesive qualities, which enable the patch preparation to stick to the gingival mucosa. Because of its mucoadhesive properties due to its high positive charge density, chitosan is a perfect polymer for delivering medications to mucosal tissues. Chitosan can also promote tissue repair and have a positive effect on human cells.[9] Natural substances that are widely available include chitin and chitosan. The shells of many molluscan creatures, like the blood clam (*Anadara granosa*), contain chitin. Relatively high levels of chitosan deacetylation, up to 91.7%, have been found in chitin derived from blood clam (Anadara granosa) shells.[10]

Additional materials are required as active ingredients in the patch fabrication process in order to maximize the gingiva's anti-inflammatory and antibacterial properties.[11] The peel of the rambutan fruit (*Nephelium lappaceum L.*) is another substance that can be employed as an active ingredient. It has antibacterial, anti-inflammatory, and corilagin, geraniin, and ellagic acid properties, as well as flavonoids, phenols (polyphenols), and saponins.[12] In Indonesia, rambutan fruit peel is a common waste product from natural resources.[13] It is still infrequently used as a therapeutic component, nevertheless. A combination of rambutan fruit peel extract (*N. lappaceum L*) and mucoadhesive gingival patches based on chitosan from blood clam shells (*Anadara granosa*) as an adjuvant therapy for inflammatory gum disease motivates researchers to investigate the impact of these patches on leukocyte counts in Wistar rats (*Rattus novergicus*). This study aimed to examine the impact of administering rambutan (*N. lappaceum L.*) peel extract in chitosan mucoadhesive patches made from blood clam shell waste as a treatment for gingivitis on the total leukocyte cell count in Wistar rats (*R. novergicus*).

MATERIALS AND METHODS

The Brawijaya University Research Ethics Committee granted research approval for the use of white rats (Rattus norvegicus) as experimental animals through a certificate of ethical license, number 073-KEP-UB-2021. Post test only randomized control group design research is used in this true experimental in vivo investigation. The simple random sampling method was employed to pick samples for this investigation, which included five treatment groups. Male, healthy Wistar rats (*R. norvegicus*) between the ages of two and three months, weighing between 100 and 200 grams, served as the study's test subjects. The rats in this study were split up into two control groups and three treatment groups. The study was carried out at the following locations: Materia Medica Laboratory Batu, Industrial Research and Consultation Centre Surabaya, Hartono Medika Laboratory, Healthy Animal Clinic Laboratory, Animal House Faculty of Dentistry at Brawijaya University, and Oral Biology Laboratory of the Faculty of Dentistry at Brawijaya University.

Measurement cups, rotary evaporators, ovens, Arlen Meyers, beakers, Buchner filters, pipettes, blenders, 100 mesh sieves, handscoops, masks, micropipettes, glass beakers, aluminum foil molds, surgical blade no. 15, micromotors, separating disc bur, tweezers, tiny plastic containers, microtomes, paraffin molds, deck glass, object glass, and light microscopes are among the equipment and supplies utilized. Research Materials: ethanol 96%, distilled water, blood clam shells (Anadara Granosa), ethanol 70%, ethanol 80%, ethanol absolute, xylol, *Porphyromonas gingivalis* in the preparation of lipopolysaccharide, chloroform, buffered neutral formaline 10%, liquid paraffin, ethanol 70%, ethanol 80%, ethanol 96%, and distilled water.

Blood clam shells (*A. Granosa*) and rambutan peel extract (*N. lapaceum L*) are combined to create a chitosan-based patch known as a chitosan mucoadhesive patch. The maceration procedure uses a 96% ethanol solvent as the active component. Unit Pelaksana Teknis (UPT) Materia Medica Batu conducted an identification test on rambutan peel, Islamic State University of Maulana Malik Ibrahim Malang's pharmaceutical laboratory performed a Fourier-transform infrared spectroscopy (FTIR) test on blood clam shell chitosan, and Brawijaya University's Faculty of Dentistry's oral biology lab conducted a patch preparation test.

Animal House at Brawijaya University's Faculty of Dentistry. The rats were housed in a wire-covered cage made of a plastic box with a wood chaff foundation. Subsequently, physical, behavioral, and dietary observations were taken regarding the rats' overall health. LPS Pg was utilized to cause gingivitis in the gingival sulcus of the rat mandibular incisor of treatment groups 1 (KP1), 2 (KP2), and 3 (KP3) after the test animals had been acclimated for seven days. Ten-microliter pipette for a single induction on Wistar rats once a day for two days was used. On the third day following induction, the rat gingiva was inspected. Clinical signs of gingivitis in rats included redder, puffier, and easily bleeding gingiva when softly touched.

Chitosan mucoadhesive patch application on the first day following the beginning of gingivitis in rats, a patch was

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administered to the treatment group using a combination of rambutan peel extract. The patch was applied by positioning it on the rat's mandibular incisor teeth's gingival area. For six days, patches were placed twice a day, either during the day or at night. On the seventh day following patch therapy, five rats per group were put to sleep, and the gingival tissue was removed for histological preparations. In addition, a 400x magnification was used to count the leukocyte cells on the preparations to determine the average number by viewing the preparations in four different fields of view.

RESULTS

Figures 1 and Figure 2 of the KP1, KP2, and KP3 treatment groups showed leukocyte cells in the control group (-) and (+). Following hematoxylin-eosin (HE) staining, preparations were seen under a 400x magnification microscope. Leukocyte counts rose in the control group (+), suggesting an inflammatory reaction. It can be seen in the picture above that the positive control group K (+) shows the total number of leukocyte cells. The total number of

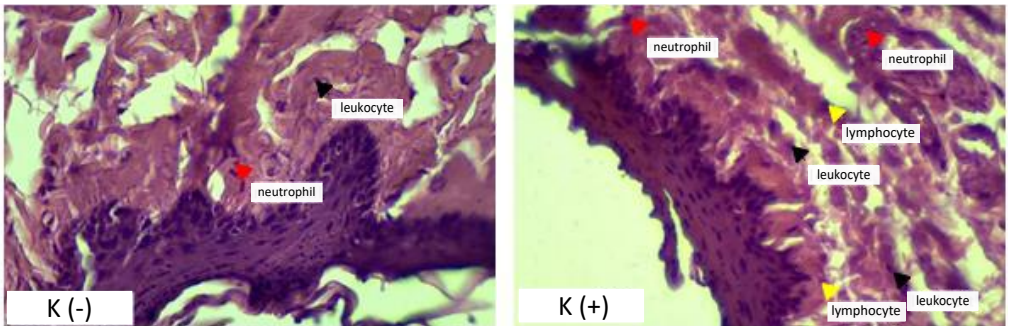

Figure 1. Leucocyte cell number on group K(-) and K(+).

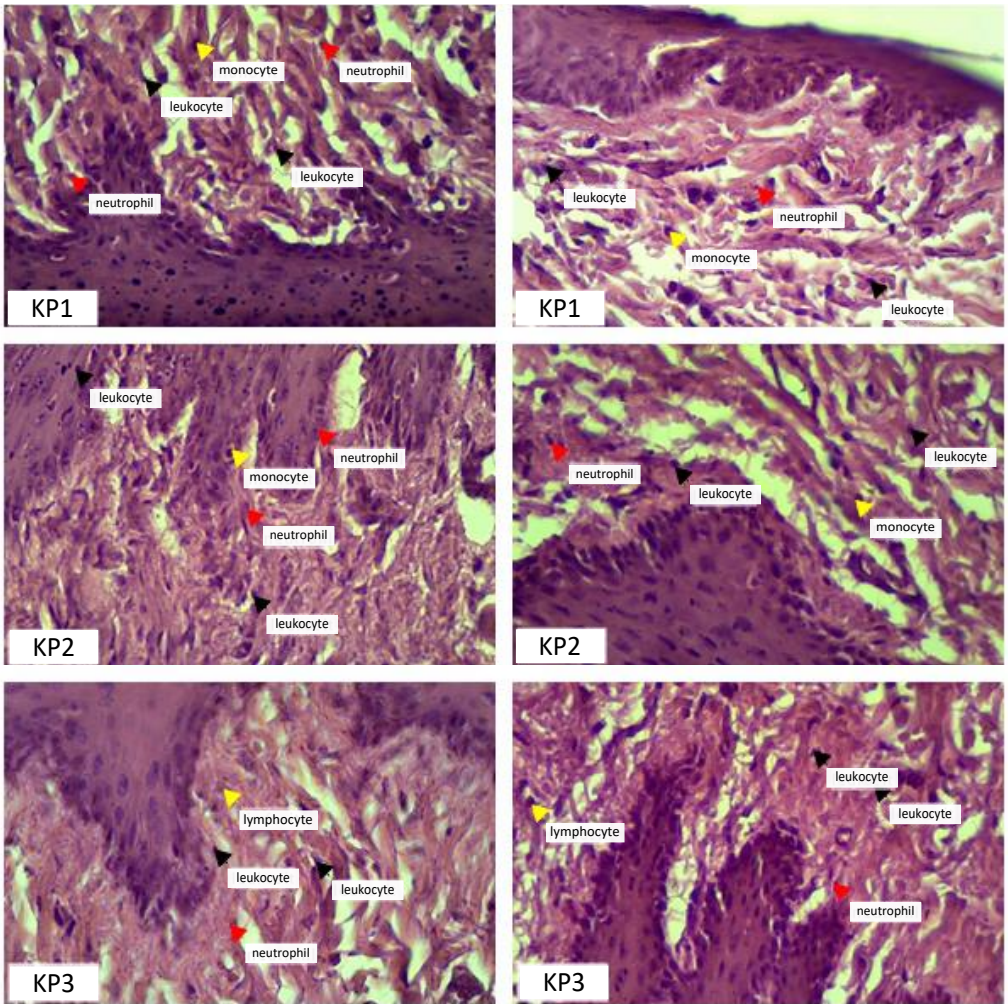

Figure 2. Leucocyte cell number on group KP1, KP2, KP3.

leukocyte cells is the least in the negative control group K (-) and treatment groups 2 and 3 (KP 2 and KP 3).

The positive control group K (+) had the largest average total number of leukocyte cells—ten—when compared to the other four groups (Figure 3). In addition, treatment groups 2 and 3 (KP2 and KP3) have the lowest average total leukocyte cell count, at seven. At four, the average number of leukocytes is the lowest in the negative control group K (-). The average number of leukocyte cells in Group Treatment 1 (KP1) is a little less than that of the K+ group, which is nine. To ascertain whether leucocyte cell count data were normally distributed, the Shapiro-Wilk normality test was used (Table 1), with a significance level of 0.05 (p>0.05). Then, data proceeded using the Kruskal-Wallis non-parametric test. The Mann-Whitney test was then performed. The Kruskal-Wallis test is used to further analyze the data since the normality test findings indicate that the data pertaining to the total number of leukocyte cells is not regularly distributed (Table 2).

The mean rank value, or the highest average ranking value in the K (+) group, is 21.90, according to the Kruskal-Wallis test results. In the meantime, the KP3 group's low

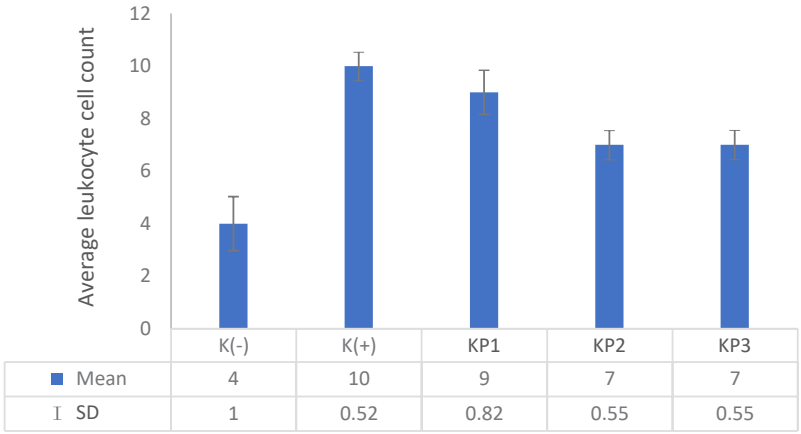

Figure 3. Diagram of average total leukocyte cell count.

Table 1. Results of the normality test between groups

	Groups	*Shapiro-Wilk*		
		Statistic	df	Sig
Total count leukocyte cells	K(-)	552	5	0.000
	K(+)	684	5	0.006
	KP1	881	5	0.314
	KP2	684	5	0.006
	KP3	684	5	0.006

Table 2. Ranking results of Kruskall-Wallis test in each group

Ranks			
	Groups	N	Mean Rank
Total count leukocyte cells	K (-)	5	3.20
	K(+)	5	21.90
	KP1	5	18.70
	KP2	5	12.50
	KP3	5	8.70
	TOTAL	25	

Tabel 3. Kruskall-Wallis Test Statistics between groups

Test Statistics	
	Total Lekocyte Cell Count Calculation
Kruskall-Wallis	21.561
Df	4
Asymp.Sig	0.000*

Note: *significant at p<0.05

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Table 4. Mann-Whitney Test comparison between groups

	Comparison of Groups		Value P
Calculation of the Total Number of Leukocyte Cells	K(-)	K(+)	0.006*
		KP1	0.007*
		KP2	0.006*
		KP3	0.011*
	K(+)	KP1	0.118
		KP2	0.007*
		KP3	0.007*
	KP1	KP2	0.021*
		KP3	0.008*
	KP2	KP3	0.058

Note: *significant at p<0.05

mean rank value is 8.70; It is evident that the average rank obtained decreases with increasing concentration of mucoadhesive patches. Thus, it can be said that the action of the concentration of the active elements of rambutan peel extract and chitosan mucoadhesive patches on the overall number of leukocyte cell counts varies between groups. The inflammatory reaction will decrease with increasing concentration. The quantity of leukocyte cells in the gingival tissue is decreasing, indicating this. Table 3, which displays the findings of statistical testing, displays the hypothesis that has been established. Asymp. It can be concluded that there is a significant difference between the control groups K (-), K (+), KP1, KP2, and KP3 based on the sig. (p-value) of 0.000 (p=0.000), which indicates that the significance value obtained is smaller or equal to 0.05 (p≤0.05).

According to the findings of the Mann-Whitney test (Table 4), comparisons between groups K (-) and K (+), KP1, KP2, and KP3 were done; each had a significant value or p-value below 0.05 (p<0.05), indicating that Ho was not accepted. Then, since the p-value is higher than 0.05 (p > 0.05), which indicates that there is no statistically significant difference between the K (+) and KP1 groups, Ho is accepted in the comparison between the two groups. Ho is rejected, suggesting that there is a significant difference between the K(+) group and the KP2 and KP3 groups, even though the p-value is less than 0.05 (p < 0.05) in the comparison between the K (+) group and the KP2 and KP3 groups.

When comparing groups KP1 and KP2 to KP3 and KP2 to KP3, the p-value is less than 0.05 (p<0.05). This suggests that there are considerable differences between groups KP1 and KP2, KP3 and group KP2, and group KP3. The positive control group K (+) and the KP2 and KP3 groups differ significantly, per the results of the Mann-Whitney test. This illustrates how the total number of leukocytes in the gingival tissue of Wistar rats is considerably decreased when rambutan peel extract (*N. lapaceum L*) and high quantities of active components are present in chitosan mucoadhesive patches.

DISCUSSION

Mucoadhesive patches can be used for therapy since they can be applied to the gingiva and kept there for a long time. Their high efficacy, efficiency, and flexibility make them the most susceptible to gingivitis.[14] Chitosan, a patch-based substance, has a great ability to interact with oral mucosal epithelial cells and extend their duration in the oral cavity. Furthermore, chitosan has a well-established, low toxicity, non-antigenic, biocompatible, and biodegradable nature.[15]

The active component, rambutan peel, has anti-inflammatory properties that can inhibit tumor necrosis factor-alpha (TNF-α).[13] LPS-induced RAW 264.7 cells, rambutan peel extract may significantly reduce NO (nitric oxide) production and control iNOS mRNA levels. The antibacterial properties of rambutan peels include flavonoids, ellagic acid, corilagin, geraniin, saponins, tannins, and polyphenols, according to the findings of phytochemical tests.[13,16]

Leukocytes play a key role in the inflammatory response, which provides powerful and quick defense against infectious illnesses and external objects. Immune system cells enter the tissues through the bloodstream to combat infection. The leukocytes then move to the infection location after entering the connective tissue. As the initial leukocyte type to reach the infection site, PMNs release chemical mediators such as pro- and anti-inflammatory cytokines.[17]

In gingivitis, elevated levels of the proinflammatory cytokine TNF-α are strongly associated with immunological response and tissue damage. Proinflammatory cytokines enhance phagocytes' ability to kill bacteria by attracting more innate cell populations to the infection site and focusing subsequent targeted immune responses on invasive microorganisms.[18] Anti-inflammatory cytokines, on the other hand, stop the infection process. Interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α) are three proinflammatory cytokines that seem to play a major part in the deterioration of periodontal tissue. It seems that IL-10's main purpose is to suppress the immune system and have anti-inflammatory effects in general, especially via preventing macrophage activity.[19] Leukocyte counts decline as a result of a reduction in proinflammatory cytokine levels. Reduced tissue inflammatory response is indicated by a drop in leucocyte cell count; gingiva can revert to normal if leucocyte activity to reduce infection is decreased.[20]

The results of the 5% concentration patch, or control group 1, were not sufficiently effective in lowering the number of leukocyte cells in comparison to the positive control group, according to the Mann-Whitney test results obtained to obtain significant differences between control groups. This is because rambutan peel's phenolic component

level is insufficient to lessen gingival irritation. According to research by Nurrohman et al. (2021), a formula with a higher concentration is required for the active ingredients to function optimally, as the 5% extract of the active ingredient can only function as a minimum concentration to inhibit the growth of pathogens that cause inflammation in the oral cavity. However, it was discovered that the results of the two concentrations were significant at 10% and 15% in comparison to the positive control group because rambutan peel had a higher level of phenolic compounds, making it ideal for lowering gingival inflammation.[21] Sarmira et al. (2021) claim that the extract's antibacterial active ingredients content increases with extract concentration.[22]

In order to be used as an additional treatment for inflammatory gum disease, chitosan mucoadhesive patches in conjunction with rambutan fruit peel extract (Nephelium lappaceum L) can successfully lower the total number of leukocyte cells in the gingival tissue of white rats (Rattus norvegicus) injected with LPS P. gingivalis.

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