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The number of lymphocytes in rabbit gingival wounds covered with a periodontal pack with lime peel extract

Malianawati Fauzia[1*], Ega Lucida Chandra Kumala[1], Ayu Nabilatul Ummah[2]

[1]Department of Periodontics, Faculty of Dentistry, Universitas Brawijaya, Malang, Indonesia;
[2]Undergraduate Degree in Dental Medicine, Faculty of Dentistry, Universitas Brawijaya, Malang, Indonesia;

*Corresponding author:
Malianawati Fauzia;
Department of Periodontics, Faculty of Dentistry, Universitas Brawijaya, Malang, Indonesia;
meli_fkg@ub.ac.id

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ABSTRACT

Introduction: Periodontal packs have frequently received extra anti-inflammatory and antibiotic medications, but they might trigger allergic reactions. As a result, alternative materials are required, such as herbal plants like lime peel, which can hasten wound healing without producing negative side effects. Citrus aurantifolia Swingle's peel extract, which has active ingredients with anti-inflammatory, antioxidant, antimicrobial, and antibacterial actions, can hasten the healing of wounds when added to periodontal packs. One marker in the healing process is reducing the production of lymphocytes. This study aims to determine the effect of lime (*Citrus aurantifolia* Swingle) peel extract on periodontal packs and the number of lymphocytes in rabbit gingival preparations.

Methods: Thirty-two New Zealand white rabbits were employed as the sample; they were harmed on the mandibular gingiva using a punch biopsy with a diameter of 2 mm. The animals were split into eight groups on the third and fifth days, with the treatment group receiving a periodontal pack containing lime (*Citrus aurantifolia* Swingle) peel extract and the control group receiving a periodontal pack without it. In order to count the lymphocytes, histological preparations were examined using HE staining.

Results: Statistical test results showed a significant difference in the number of lymphocytes between the control group and the treatment group on days 3 and 5 (ANOVA, $p < 0.05$).

Conclusion: Lime (*Citrus aurantifolia* Swingle) peel extract can decrease the number of gingival lymphocytes in rabbit's periodontal tissue.

Keywords: Lime (*Citrus aurantifolia* Swingle) peel extract, Periodontal pack, Lymphocytes, Rabbit Gingival.
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INTRODUCTION

Periodontal disease is common in developed and developing countries and affects around 20–50% of the global population.[1] Periodontitis, one of the periodontal diseases, has a prevalence of 67.8% in people aged 15 years in Indonesia, which means that one in every ten Indonesian residents has periodontitis.[2] Periodontal disease ranging from moderate to severe requires surgery.[3] Surgery aims to clean or eliminate disease, restore anatomical condition, and restore function.[4] Among the surgical procedures is the use of a periodontal pocket. Using a periodontal pack has many advantages, including reducing the chance of postoperative problems, including wound infection and bleeding, speeding up tissue healing by reducing physical trauma from chewing and speaking, and decreasing the growth of granulation tissue.[5]

The commonly used periodontal pack materials are zinc oxide, eugenol, and non-eugenol base materials. Non-eugenol periodontal packs are one of the most widely used in this era compared to eugenol-based use. It is because eugenol was discovered to irritate oral mucosal tissue, produce allergic reactions, and promote tissue necrosis, especially in bones, which delays the healing of wounds.[6] However, the non-eugenol periodontal pack is a dimensionally unstable material that causes contractions for 1 minute after its placement, resulting in delayed healing.[7] The two periodontal packs mentioned above lack therapeutic qualities; tissue healing is only accomplished by avoiding physical injury during chewing and speaking, preventing the growth of granulation tissue, and minimizing postoperative discomfort.[5] However, in this case, the non-eugenol periodontal pack tends to have non-irritant properties, so it is used more frequently.[6] Examples of non-eugenol periodontal packs are COE-packs and Baer formulas. In this study, the Baer periodontal pack formula will be used because it has the same original composition without the addition of a mixture of other ingredients, unlike COE packs, which use a mixture of other ingredients.[8]

Wound healing occurs in several phases, including the inflammatory phase. The inflammatory phase occurs from the initial wound formation until the fifth day. The existence of inflammation in wound healing must be inhibited because it can cause abnormal wound healing, resulting in pathological inflammation that can lead to more severe complications and chronic inflammation. In this inflammatory phase, there are various inflammatory cells, including

lymphocytes. The role of lymphocytes in wound healing is to release lymphokines (IFN-y). Lymphokines strongly influence the inflammatory process, influencing macrophage aggression and chemotaxis in wound healing. After day 3, lymphocytes are visible in the wound and are thought to play a crucial role in regulating wound healing by producing the extracellular matrix scaffold and remodeling collagen. Experimental investigations demonstrate that inhibiting T cells reduces the strength of the wound and impairs collagen synthesis. On day 5, the lymphocytes increased; the decrease in inflammatory cells was a sign of the end of the inflammatory phase and could continue to the next phase.[9]

Alternative materials, including herbal plants like lime, are required to speed up wound healing without having negative side effects. Anti-inflammatory and antibiotics are frequently added to the periodontal pack. However, doing so might induce allergic reactions.[10] Apart from fruit, the lime peel has medicinal properties. The ingredients in the lime peel are alkaloids, flavonoids, tannins, and saponins, which can inhibit the effectiveness of bacterial growth and accelerate wound healing and have anti-inflammatory, antioxidant, antimicrobial, and antibacterial properties effects.[11,12] The advantages of using lime peels are that they are easy to obtain, relatively inexpensive, cause common side effects compared to synthetic drugs can reduce lime peel waste.[11,13] Lime peel waste accounts for 50-65% of the total weight of the residue. This waste can give off a bad smell and cause environmental pollution. So that if lime peel waste is used properly, it can minimize environmental pollution.[14] Ability of lime peel extract as a wound healer has been widely studied. As with the research, it has been proven that the effectiveness of healing cuts.[11]

Periodontal packs must have a soft texture but sufficient plasticity and flexibility to facilitate application in the oral cavity. Therefore, a mixture of lime peel extract and a periodontal pack must be suitable. This suitability will result in homogeneous periodontal pack preparations, namely with extract concentrations of 5%, 10%, and 15%, according to previous studies that obtained homogeneous periodontal pack dough that has high effectiveness and is not easily brittle.[10,15]

Based on the reasons above, the current periodontal pocket has its drawbacks. For this reason, lime peel contains anti-inflammatory, antioxidant, antimicrobial, and antibacterial potentials that are important in accelerating wound healing. There has not been any study up to this point that examines the impact of lime peel extract in periodontal packs on the number of lymphocytes in rabbit gingiva. As a result, the authors are curious to find out how lime extract affects the number of lymphocytes in rabbit gingival preparations.

METHODS

This study will employ laboratory, *in vivo*, and true experimental methods. A Post Test Only Control Design was used for the research to determine the effect of lime (*Citrus aurantifolia* Swingle) extract on the periodontal pack on the number of lymphocytes in rabbit gingival preparations.

The subjects were 32 male rabbits divided into 8 treatment groups, and samples were selected randomly. K1 and K2 are the sample groups with no addition of lime peel extract to the periodontal dressing; P1 and P4 are the sample groups with 5% lime peel extract addition to the periodontal dressing; P2 and P5 are the sample groups with 10% cocoa pod extract addition to the periodontal dressing; P3 and P6 are the sample groups with the addition of 15% lime peel extract to the periodontal dressing. Each group with the same percentage of extract dose underwent different experimental observation times. Groups K1, P1, P2, and P3 were observed on the third day after the treatment started, then groups K2, P4, P5, and P6 were observed on the fifth day.

Sample

The sample used for this study were the male New Zealand White rabbits, aged 4-5 months and weighing 3–4 kg. The sample was chosen because it has a high potential to be used as an object for laboratory research. After all, it has functions, physiological features, and structures in the periodontal tissue that are very similar to those in humans, especially in the structure of the gingival mucosa.

Research Procedure

After selecting rabbits that meet the criteria and before the rabbits are operated on, they will be left to adapt in the lab for seven days. After seven days, the rabbit will undergo periodontal surgery. The first thing to do is apply an antiseptic solution containing 70% alcohol to the area to be operated. Furthermore, the rabbits were anesthetized intramuscularly with ketamine and xylazine to provide a sedative response and an analgesic effect. The dose of ketamine is 25 mg/kg, and the dose of xylazine is 3 mg/kg to strengthen the anesthetic response. Periodontal surgery in rabbits using a punch biopsy with a diameter of 2 mm on the mandibular gingiva. The depth must reach the alveolar bone of the mucosa attached to the gingiva on the labial side beneath the incisor region. To avoid defects in the bone, deep surgery on the alveolar bone does not damage the alveolar bone in rabbits that have been treated with anesthesia. Furthermore, the wound was cleaned with two solutions, namely, 0.9% NaCl solution and 0.9% H2O2 solution.[10,16]

Gingiva of rabbits that had undergone periodontal surgery, causing open wounds. The gingival wound that formed was covered with a periodontal pack. The control group used a periodontal pack without lime peel extract, while the treatment group added lime peel extract. Apply the periodontal pack to the treated area until it covers the entire wound surface, then press gently with an excavator. Furthermore, sewing was done with 5.0 silk thread sewing material and a simple interrupted sewing technique.[17]

Making a periodontal pack using the Baer formula, which can be obtained by mixing powder and paste, Powder preparation is done by mixing 28.5 grams of rosin with 21.5 grams of zinc oxide and mixing until homogeneous. Next, make the paste by stirring the hydrogenated fat and zinc oxide until homogeneous. Use 47.5 g of hydrogenated fat and 2.5 g of zinc oxide. After each powder and paste ingredient has been prepared, the two ingredients are mixed with the same amount of 50 mg so that a mixture of 100 mg is obtained.

Table 1. Division of addition of lime peel extract to Baer's formulation of the periodontal pack.

	Group	Baer's formulation of the periodontal pack (mg)	Lime peel extract (mg)
Day-3	K1 0%	100	0
	P1 5%	95	5
	P2 10%	90	10
	P3 15%	85	15
Day-5	K2 0%	100	0
	P4 5%	95	5
	P5 10%	90	10
	P6 15%	85	15

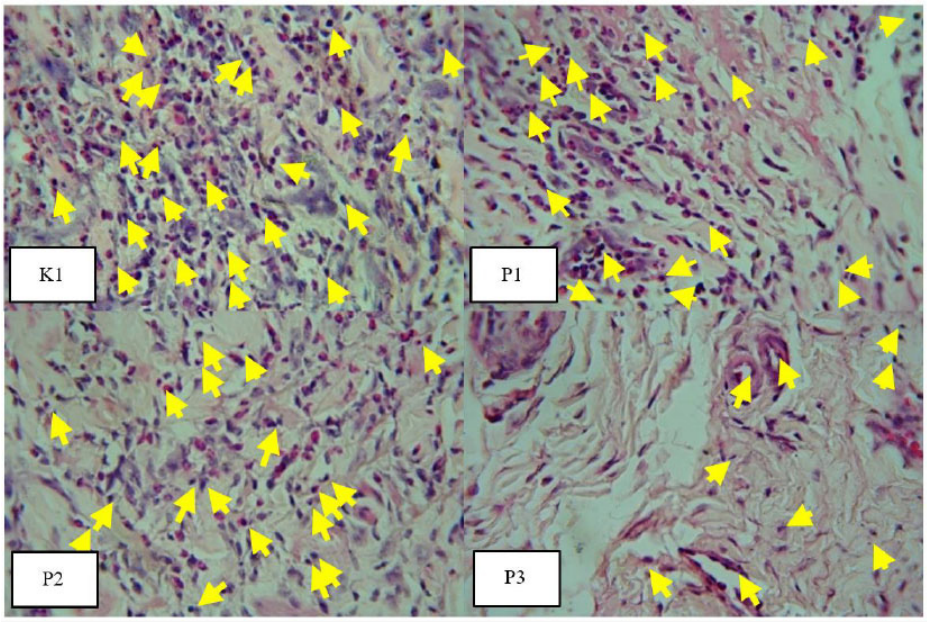

Figure 1. Lymphocytes prepared on day 3 of HE staining, magnification 400x (source: research documentation).

Stir the two little by little until the dough is homogeneous.[16] Then, mix the lime peel extract into the periodontal pack mixture according to the dosage (Table 1).

On the third and fifth days, tissue samples from each experimental group were taken to count the number of lymphocyte cells in the gingival granulation tissue. Furthermore, ketamine was injected intramuscularly at 200 mg/kg body weight to decapitate the test animals while under anesthesia. After being acquired, the gingival granulation tissue is subsequently put in a container with a 10% formalin solution. Hematoxylin and eosin staining was used to prepare tissue samples. A binocular microscope with 400x magnification and 5 separate fields of view was used to observe fibroblast cells. The assumption test obtained homogeneous and normal results with the Shapiro-Wilk and Levene tests, followed by a one-way ANOVA, post-hoc Tukey, and independent T-test.

RESULTS

The observations in this study used a microscope to view lymphocyte cells, which were counted manually with the Imagej application in as many as 5 different fields of view for each sample. The study results on average lymphocyte cells in rabbit gingival preparations on day 3 can be seen in Figure 1, and day 5 can be seen in Figure 2.

Table 2 and Figure 1 show that the number of lymphocyte cells was lower in the treatment groups P1, P2, and P3 compared to the control group (K1). Of the four groups, it is shown that the highest lymphocyte count was in the control group (K1) at 24.8, and the lowest was 17.05 in the P3 group.

Then, from Table 2 and Figure 2, the same thing is obtained. The four pictures from each group on day 5 showed a decrease in lymphocytes in the P4, P5, and P6 treatment groups compared to the control group (K2). The highest number of the four groups was in K2, with a score of 27.45, and the lowest was in P6, with an average of 14.1. A clearer comparison of the average number of lymphocytes in the gingival preparations of rabbits from each group can be seen in the graph in Figure 3.

Based on the graph in Figure 3 below, on the third and fifth days, it was seen that the group without giving lime peel extract and the group giving lime peel extract at doses of 5% and 10% experienced an increase from day 3 to day 5, while it decreased with the administration of 15% lime peel extract. It shows that the higher the concentration of lime peel, the lower the average number of lymphocytes.

The number of lymphocyte cells on histological preparations with a 400x magnification and as many as 5 separate fields of view for each sample was counted, and 32 rabbits divided into 8 groups were seen. Following the lymphocyte cell count calculation, the one-way ANOVA test was used to analyze the data (Table 2). Before that, a normality test was carried out by carrying out the Shapiro-Wilk test, which showed significance ($p>0.05$), meaning that the study data were normally distributed. Furthermore, the homogeneity test used the Levene test to obtain a significant ($p>0.05$) result, meaning the data was homogenous.

The test using one-way ANOVA obtained a significance level of 0.000 or 0.05. By obtaining these results, the data can be said to have a significant effect of lime peel extract (*Citrus aurantifolia* Swingle) in the periodontal pack on the number of lymphocytes in the rabbit gingival preparations. With this, the test is continued with the post-hoc Tukey test, which is carried out separately between

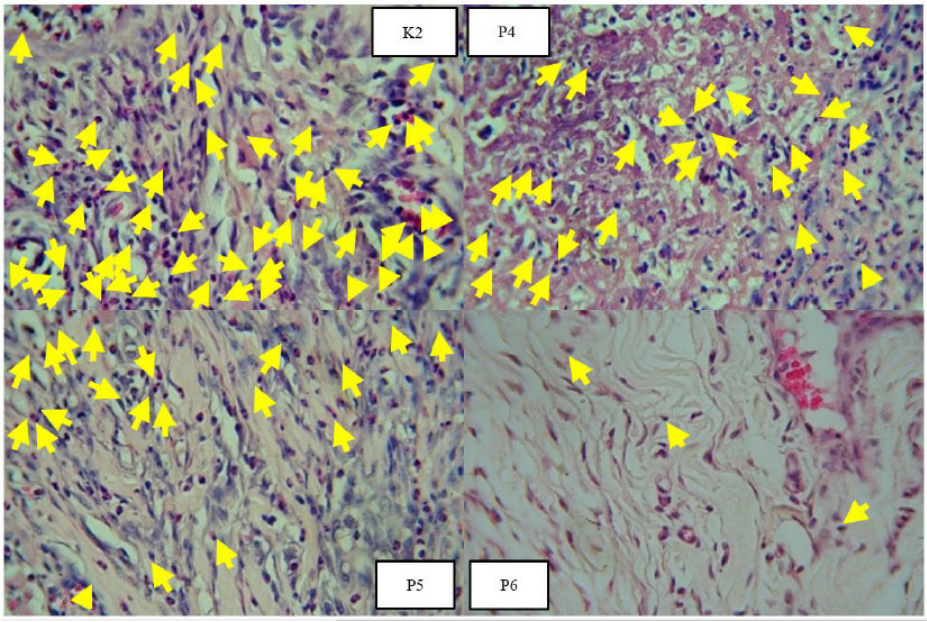

Figure 2. Lymphocytes prepared on day 5 of HE staining, magnification 400x. (Source: Research Documentation).

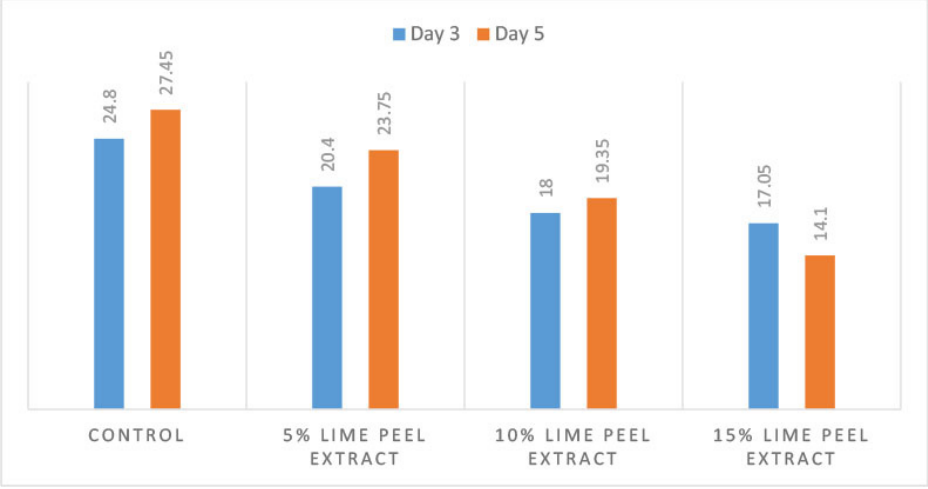

Figure 3. Graph the average number of lymphocytes prepared from rabbit gingiva in each group.

observation times to find out from the umpteenth group whether there is a difference from day to day.

In this test, the data indicated a significant difference if the P value was less than 0.05 and not significant if it was greater than 0.05. Post-hoc Tukey test results significantly differed between groups K1 and P1, K1 and P2, K1 and P3, P1 and P2, and P1 and P3. In comparison, the difference was not significant between the P2 and P3 groups.

The test results in the fifth-day table (Table 5) comparing each group with the overall comparison obtained a $p<0.05$, which stated that there was a significant difference in the number of lymphocytes in the gingiva of rabbits in all groups compared with the other groups on day 5.

An independent T-test was performed to see whether there was a difference in the number of lymphocyte cells between observation times. The independent T-test results at each dose between the third and fifth days of observation were taken from Table V above with a significance level of 0.05. The groups that did not receive lime peel extract and those that received 5% and 10% lime peel extract both exhibited an increase in the table. Meanwhile, the administration of 15% lime peel extract decreased, so it can be concluded that a 15% dose of lime peel extract (Citrus aurantifolia Swingle) affected significantly decreasing the number of lymphocytes.

DISCUSSION

This study used New Zealand White rabbits because a previous study by Shantiningsih et al. (2013) concerning the increase in the Number of Micronuclei in the Gingival Mucosa of Rabbits After Panoramic Radiography Exposure proved that there are similarities in the structure of the gingival mucosa and micronuclei between humans and rabbits, so that rabbits have a high potential for being used as an object of laboratory research and have a function, physiological, and structural features of the periodontal tissue that are very similar to that of humans.[18]

Periodontal packs must have a soft texture but sufficient plasticity and flexibility to facilitate application in the oral cavity. Therefore, a mixture of lime peel extract and the periodontal pack must be suitable to produce a homogeneous periodontal pack preparation, namely with an extract concentration of 5%, 10%, and 15%, according to previous studies that obtained a homogeneous periodontal pack mixture with high effectiveness and not easy brittleness.[10,15]

Adding a periodontal pack that is often done can cause allergic reactions, so it requires ingredients without causing side effects, one of which is using herbal plants, namely lime peel. Alkaloids, flavonoids, tannins, and saponins are among the active components of lime peel extract (*Citrus aurantifolia* Swingle). These active substances may increase the efficiency of wound healing.[10,11] Alkaloid compounds act as antibacterials; tannins have antimicrobial properties; saponins play an anti-inflammatory role; and flavonoids act as antimicrobials, anti-inflammatories, and antioxidants. By inhibiting the activity of inflammatory cells like neutrophils and macrophages, as well as the expression and production of inflammatory mediators like COX-2, PGE2, ROS, TNF, and IL-6, saponins and flavonoids act as anti-inflammatory agents. Additionally, they act as antioxidants by binding to free radicals produced by Th1 cells (CD4+ T

cells) to prevent damage.[12] Furthermore, saponins can hasten TGF synthesis, which T lymphocytes release.TGF- acts as the primary signal for regulating fibroblasts, reducing the number of lymphocytes while increasing the number of fibroblasts and hastening the inflammatory phase, antibacterial and anti-inflammatory effects work together during the inflammatory process. They collaborate to reduce the effects of excessive inflammation, allowing the inflammatory phase to be brief. The healing phase is then followed by a proliferative phase, which is characterized by increased fibroblast activity in the synthesis of collagen fibers.[10,19] According to Krismaya et al. (2019), the ingredients stored in lime peel extract stated that saponins were 3.05%, flavonoids were 2.78%, tannins were 2.14%, and alkaloids were 1.86%.[12]

Table 2. **One Way ANOVA test results.**

Observation	Group	Mean	F	p
Day 3	K1	24.80	58.853	0.000
	P1	20.40		
	P2	18.00		
	P3	17.05		
Day 5	K2	27.10	159.843	0.000
	P4	23.75		
	P5	19.35		
	P6	14.10		

Table 3. **Post Hoc Turkey test results on Day 3.**

Day 3 Group	K1	P1	P2	P3
K1		0.000	0.000	0.000
P1	0.000		0.002	0.000
P2	0.000	0.002		0.449
P3	0.000	0.001	0.449	

Table 4. **Post Hoc Turkey test results on Day 5.**

Day 5 Group	K2	P4	P5	P6
K2		0.000	0.000	0.000
P4	0.000		0.000	0.000
P5	0.000	0.000		0.000
P6	0.000	0.000	0.000	

Table 5. **Independent t-test results.**

Concentration	Observation Time	Group	Mean	p	Decision
Control	Day 3	(K1)	24.80	0.000	Significant
	Day 5	(K2)	27.45		
5% lime peel	Day 3	(P1)	20.40	0.000	Significant
	Day 5	(P4)	23.75		
10% Lime Peel	Day 3	(P2)	18.00	0.030	Significant
	Day 5	(P5)	19.35		
15% lime peel	Day 3	(P3)	17.05	0.000	Significant
	Day 5	(P6)	14.10		

According to Izzaty et al. (2017) and Singh et al. (2017), lymphocytes are one of the body's defense cells that optimally play a role in the chronic phase. Lymphocytes could be detected in the wound tissue on days 3 and 5, and their numbers increased. An increase in lymphocytes occurs in chronic inflammation as a humoral and cellular response. In addition to an increase in lymphocytes, the fifth day in the wound tissue is the end of the inflammatory phase. This inflammatory phase activates lymphocytes, which are directly related to wound healing. Lymphocytes and macrophages interact in a bidirectional manner, which plays an important role in the propagation of chronic inflammation. Lymphocytes will attract antigens, resulting in the activation and release of lymphokines (IFN-y). Lymphokines (IFN-y) is important in the stimulation and activation of macrophages for phagocytosis. Activated macrophages will release growth factors that can cause fibroblast proliferation, epithelialization, and angiogenesis to achieve wound healing.[9,20]

Histologically, for calculations, lymphocytes will appear on the preparation through a microscope in the form of a large, rounded nucleus with a single nucleus and a purple color; the cytoplasm appears to encircle the light blue nucleus slightly. Lymphocytes can be observed on the third and fifth days. This statement is in accordance with Singh et al. (2017), who found that lymphocytes are detected from 72 to 120 hours, meaning that lymphocytes can be observed in the wound tissue from the third to the fifth day.[20] In a study by Izzaty et al. (2017), it was stated that lymphocytes increased on the fifth day.[9] Therefore, this study calculated the number of lymphocytes in the gingiva of rabbits on days 3 and 5 through a microscope. Overall calculations show that the treatment group given lime peel extract on each third and fifth day could reduce the number of lymphocytes

along with the additional dose of lime peel extract compared to the control group due to the presence of anti-inflammatory ingredients in lime peel causing a decrease in the number of lymphocytes, which is a sign of the end of the inflammatory phase of the process and can continue into the next phase. This condition is also in line with the study of Izzaty et al. (2017), which stated that the anti-inflammatory content in the honey extract could reduce the number of lymphocytes in wound healing during the inflammatory phase. Although the extract ingredients and active substances differ from those found in lime peels, both contain active anti-inflammatory substances.[9] This was reinforced by Utama et al. (2014), who used papaya leaf extract containing the same active substances, alkaloids, saponins, and flavonoids as anti-inflammatories. The results of this study concluded that the addition of papaya leaf extract could reduce the number of lymphocytes in the gingiva of the male Wistar rat.[21]

According to the one-way ANOVA test, there was a difference in the average number of lymphocytes that impacted each control and treatment group, which produced significant results with a p-value of 0.000. This experiment demonstrated that lime peel extract (*Citrus aurantifolia Swingle*) impacted the number of lymphocytes while a rabbit gingival wound healed, with the number of lymphocytes declining with each successive dose. The decrease in lymphocytes was affected by the active substance, one of which is the flavonoid in lime peel extract, which belongs to the phenol compound group with the greatest anti-inflammatory effects through inhibition of cyclooxygenase and lipoxygenase, which can limit the number of inflammatory cells such as lymphocytes in inflamed tissues. It makes the inflammatory process run faster to immediately proceed to the next phase, namely the proliferative phase. Inhibition of this cyclooxygenase can decrease prostaglandin production, resulting in reduced vascular permeability, vasodilatation of blood vessels, and reduced local blood flow, which causes a decrease in the number of inflammatory cells.[22] This situation is consistent with the findings of Izzaty et al. (2017), who discovered that administering haruan extract causes a decrease in the number of lymphocytes, indicating that the antigen has vanished, indicating that the inflammatory phase is brief and can continue into the proliferation and remodeling phases of the wound healing process.[9] According to earlier research by Fauzia et al. (2022), adding lime peel extract (Citrus aurantifolia Swingle) to the periodontal dressing impacted the number of blood vessels, which is further evidence that doing so can affect how wounds heal.[23] In addition, other researchers also stated that lime peel extract was able to accelerate the wound healing process.[24]

Based on the results of the Tukey post hoc test, which was previously carried out by the one-way ANOVA test with a 95% confidence level, it showed that the group that was not given lime peel extract was given 5% and 10% lime peel extract from the third to the third day of observation. 5 has increased. On the other hand, the administration of a 15% dose of lime peel extract decreased from day 3 to day 5. The results of the observation test on day 3 revealed a significant difference between groups K1 and P1, K1 and P2, K1 and P3, P1 and P2, and P1 and P3. There was a significant difference between the control group and the group that was given lime peel extract because of the active ingredient in the lime extract. It is similar to the results of a study by Adi et al. (2017), which showed that there was a significant difference in the amount of IL-6 expression in rats because there were anti-inflammatory substances in flavonoids that reduced the amount of IL-6 expression in the gingiva of Wistar rats that were included in the inflammatory phase.[13] Furthermore, the group between P1 and P2 showed a significant difference in the decrease in the condition due to the dose of lime peel extract reducing the average number of lymphocytes on the third day of observation. In contrast, the difference in the decrease was not significant between the P2 and P3 groups on the third day of observation because they had not reached the peak of the decline, so it is estimated that the next day with a higher extract dose, the lymphocytes will be reduced then, between the P1 and P3 groups, there was a significant difference because the difference in the dose given to the periodontal pack was high enough to cause cyclooxygenase and lipoxygenase inhibition. If there are obstacles in these pathways, the production of prostaglandins, leukotrienes, and thromboxane decreases, and the number of lymphocytes decreases because the migration of proinflammatory mediators is under pressure. It is similar to the previous study by Utama et al. (2014), which produced no significant differences in the papaya leaf extract between groups III and IV before there was a significant difference in reduction between groups IV and V, and there were significant differences in groups II and V because there was a high difference in the papaya leaf extract.[21]

Tukey's post hoc test was used on the fifth day of observation to compare the four groups (control and three treatments), and the results were significantly different. In other words, administering lime peel extract (Citrus aurantifolia Swingle) with successive doses of 5%, 10%, and 15% can reduce the number of lymphocytes on day 5. The decrease in the number of lymphocytes resulted in a significant difference. It is demonstrated by the post hoc Turkey test results, which were significant ($p<0.05$).

The independent t-test to determine whether there was a difference in the number of lymphocytes between observation times showed a significant increase in the periodontal pack group that was not given lime peel extract and the group given lime peel extract of 5% and 10%. The administration of 15% lime peel extract in the periodontal pack resulted in a significant decrease. The control group, or the group not given lime peel extract, had an increased lymphocytes count from the third to the fifth day. It may be due to the absence of an active substance in the lime peel extract, while the lime peel extract dose of 3% and 10% have increased because the dose extracted in the periodontal pack is too low so that the active substance is unable to affect the number of lymphocytes, and so it still experiences slow healing. It is normal because the increase occurred on day 5.[9] However, the 10% dose resulted in a lower average lymphocyte count than the 5% dose. Furthermore, the administration

of lime peel extract at a dose of 15% from the third to the fifth day was different from the other groups because it experienced a decrease, which indicated that the antigen had disappeared and the inflammatory phase was over so that it could continue to the proliferative phase. It is because the greater the dose of lime peel extract added to the periodontal pack, the lower the number of lymphocytes. According to Adi et al. (2017), the larger the dose, the stronger the anti-inflammatory reaction will affect the number of active substances, such as flavonoids in lime peel extract, and the greater the ability of these ingredients to inhibit inflammation.[13] Although the study did not use a periodontal pack, another study using a periodontal pack by Vavata et al. (2019) with cinnamon extract stated that of the three doses of 5%, 10%, and 15%, the 15% dose was the most effective in accelerating wound healing.[25] On gingival wounds of rabbits and by using cocoa fruit extract, which has the same properties and content as cocoa and lime peel as anti-inflammatory, antioxidant, and antimicrobial, states that a dose of 15% is effective for accelerating wound healing from 5% to 10% in the gingiva of rabbits.[10] Even though the two studies used different extract ingredients, it was reinforced by the research of Adi et al. (2017) that the larger the dose of lime peel extract, the stronger the effect.[13]

The analysis showed that the periodontal pack treatment group, which was given 3% and 10% lime peel extract, experienced an increase in the number of lymphocytes in the gingiva of rabbits. It may be due to the low active substance of the lime peel added to the periodontal pack, which is still lacking compared to the active substance contained in the administration of 15% lime peel extract, so the effectiveness of the content is not optimal in influencing the number of lymphocytes.[15] Therefore, giving the periodontal pack 15% lime peel extract decreased the number of lymphocytes. This situation is the same as that in the study by Izzaty et al. (2014), which obtained an increase in lymphocytes in 25% haruan extract from the first day to the seventh day and a decrease in lymphocytes in 50% haruan extract from the third to the seventh day.[9] This decrease is caused by the effect of the active compound content of lime peel, which is extracted in the periodontal pack according to the needs of the wound to reduce the production of lymphocytes, and inflammation can be limited, which makes healing faster.

CONCLUSION

The administration of lime peel extract (Citrus aurantifolia Swingle) to the periodontal pack can influence the reduction in lymphocytes in the rabbit gingival preparations, according to the study's findings. When employing a concentration of 15%, the reduction in lymphocytes is successful and implies quicker wound healing.

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ETHICAL CLEARANCE

The study was approved by the animal care and use committee at Brawijaya University, Malang, with the ethics committee number 102-KEP-UB-2022.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHOR CONTRIBUTIONS

MF and ELCK contribute to the study's conceptualization, literature search, clinical studies, methodology, data and statistical analysis, investigation, manuscript editing and reviewing. MF also contributes as a manuscript guarantor. ANU contributes to literature searches, clinical studies, experimental studies, data acquisition and data analysis, statistical analysis, and manuscript preparation. All authors have read and approved the final manuscript.

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