IJKG INTERDENTAL Jurnal Kedokteran Gigi

Interdental Jurnal Kedokteran Gigi
Website: https://e-journal.unmas.ac.id/index.php/interdental
ISSN 1979-9144 (print), ISSN 2685-5208 (online)

Research Article

The Effect of Aloe Vera *(Aloe barbadensis Miller)* Extract on the Number of Inflammatory Cells (Neutrophils and Macrophages) in the Gingivitis Model *(Rattus norvegicus)* Wistar Rat Model

[1]Netta Anggraini, [2]Refika Ananta, [3]Fauzia Nilam Orienty

[1] Department of Periodontics, Faculty of Dentistry, Universitas Baiturrahmah, Padang, Indonesia
[2] Undergraduate Program, Faculty of Dentistry, Universitas Baiturrahmah, Padang, Indonesia
[3] Department of Conservation, Faculty of Dentistry, Universitas Baiturrahmah, Padang, Indonesia

Received date: January 7, 2026 **Accepted date: July 27, 2026** **Published date: August 12, 2026**

KEYWORDS

Aloe vera extract, gingivitis, inflammatory cells

DOI: 10.46862/interdental.v22i2.13384

ABSTRACT

Introduction: Gingivitis is an inflammatory gingival disease associated with local factors, medication use, and malnutrition. Aloe vera (Aloe barbadensis Miller) contains flavonoids, tannins, saponins, polyphenols, and steroids with potential anti-inflammatory effects. This study aimed to determine the effect of aloe vera extract at concentrations of 50%, 75%, and 100% on neutrophil and macrophage counts in a gingivitis model of male Wistar rats.

Materials and Methods: This laboratory experimental study used a posttest randomized controlled group design involving 48 male Wistar rats divided into four groups: negative control and gingivitis groups treated with 50%, 75%, or 100% aloe vera extract. The extract was applied topically to the mandibular anterior gingiva twice daily for five days. Tissue samples were collected on days 3 and 5 and stained with hematoxylin and eosin. Data were analyzed using two-way ANOVA and Bonferroni tests ($p<0.05$).

Results and Discussion: Topical aloe vera extract significantly reduced inflammatory neutrophil and macrophage counts ($p<0.001$). On day 5, mean neutrophil counts were 0.67, 0.17, and 0 cells/mm³ in the 50%, 75%, and 100% groups, respectively. Mean macrophage counts were 1, 0.3, and 0 cells/mm³, respectively.

Conclusion: Topical aloe vera extract demonstrated anti-inflammatory activity, with 75% and 100% concentrations being the most effective in reducing inflammatory cell counts in gingivitis.

Corresponding Author:
Refika Ananta
Undergraduate Program, Faculty of Dentistry
Universitas Baiturrahmah, Padang, Indonesia
Email: refikaananta145@gmail.com

How to cite this article: Anggraini N, Ananta R, Orienty FN. The Effect of Aloe Vera (Aloe barbadensis Miller) Extract on the Number of Inflammatory Cells (Neutrophils and Macrophages) in the Gingivitis Model (Rattus norvegicus) Wistar Rat Model. Interdental Jurnal Kedokteran Gigi. 2026;22(2):291-8. doi: 10.46862/interdental.v22i2.13384

INTRODUCTION

Gingivitis is one of the most common oral health problems in society, with a very high prevalence. This disease is a periodontal disease characterized by inflammation of the gingival tissue due to the accumulation of plaque. Bacterial plaque and other local and systemic factors. Gingivitis is reported to affect a large portion of the world's population and is the second most common oral disease after dental caries, making it a significant public health problem.[1,2]

Excessive and persistent inflammatory response in the gingival tissue. Inflammation is the body's defense mechanism against harmful stimuli such as microorganisms and irritants, but if it persists, it can cause tissue damage. In gingivitis, the inflammatory process is characterized by an increase in the number of inflammatory cells, particularly neutrophils and macrophages, which play a key role in phagocytosis and the release of inflammatory mediators.[3,4]

Neutrophils are the first inflammatory cells to migrate to the site of inflammation and dominate the inflammatory response in the early phase, while macrophages play a role in further phagocytosis, secretion of cytokines, and activation of the immune response. Uncontrolled activity of these two cells can worsen the condition of gingival tissue and accelerate the progression of gingivitis to more severe periodontal disease.[5,6]

The urgency of treating gingivitis is becoming increasingly important because the disease often doesn't cause painful symptoms in its early stages, leaving many sufferers unaware of their condition. If left untreated, gingivitis can progress to periodontitis, which causes damage to the supporting tissues of the teeth, tooth loss, and decreased chewing function. This situation emphasizes the importance of early control of gingival inflammation to prevent more serious complications.[1,7]

Various previous studies have examined the use of natural ingredients as an alternative therapy. One of the anti-inflammatory properties is aloe vera (Aloe barbadensis Miller). Aloe vera is known to contain active compounds such as flavonoids, tannins, saponins, polyphenols, and steroids, which have anti-inflammatory and antibacterial activity and play a role in tissue regeneration.[8,9] Carolia and Sukohar[10] also reported that 100% aloe vera extract was the most effective at reducing the number of macrophages in the inflamed oral mucosa of male Sprague-Dawley rats.[10] In addition, Andri et al.[11] found that aloe vera extract can increase TGF-β levels, which play a role in the tissue healing process.[11]

However, there are still gaps in previous research. Most previous studies have only assessed one type of inflammatory cell, used different models of inflammation, or did not specifically evaluate gingivitis as a research object. Furthermore, there is still limited research examining the effectiveness of various concentrations of aloe vera extract on the number of neutrophils and macrophages simultaneously in gingival tissue. Therefore, this study has the advantage of examining the effect of aloe vera extract at several concentrations on two main types of inflammatory cells in a gingivitis model, so it is hoped that it can provide a stronger scientific basis for the use of aloe vera as an alternative anti-inflammatory therapy in periodontal disease.

MATERIALS AND METHODS

This research is experimental and was conducted in a laboratory using a posttest randomized controlled group design. The study aimed to assess the effect of administering aloe vera extract (*Aloe barbadensis Miller*) on the number of inflammatory cells, namely neutrophils and macrophages, in a

gingivitis model. The study population was male Wistar rats *(Rattus norvegicus)*. Male rats were selected to avoid biological variations due to hormonal cycles. The research sample was obtained using a random sampling technique, with inclusion criteria of rats aged 3–4 months, weighing 200–250 grams, and in good health. The sample size was determined using Federer's formula, (n-1)(t-1) ≥ 15, where t is the number of treatment groups (t = 4); this calculation resulted in a minimum of six replicates per group, which was doubled to 12 rats.[13] A total of 48 rats were randomly divided into four groups: a negative control group (Vaseline flava) and three treatment groups, each of which was given aloe vera extract with concentrations of 50%, 75%, and 100%, with each group consisting of 12 rats (six rats were decapitated for tissue sampling on day 3 and the other six on day 5).

The main material for this study was aloe vera leaves (*Aloe barbadensis Miller*) obtained from Teluk Bayur Village, South Padang District, West Sumatra, and which underwent an identification process at the Herbarium Laboratory of Andalas University. Experimental animals were obtained from the Pharmaceutical Laboratory of Perintis Indonesia University, Padang. The chemicals used included 96% ethanol as a solvent, Vaseline flava as a thickening agent, buffered formalin 10%, nitric acid 25%, hematoxylin-eosin, xylol, Canada balsam, and ketamine as an anesthetic agent. The tools used include a blender, Erlenmeyer flask, rotary evaporator, water bath, microtome, light microscope, mouse cage, silk ligature 3.0, and other histopathology equipment.

Aloe vera extract was made using the maceration method. The aloe vera flesh was air-dried, ground using a blender, and then soaked in 96% ethanol for 72 hours at room temperature. The maceration filtrate was then prepared. Evaporated using a rotary evaporator and then concentrated using a water bath until a thick extract was obtained. The extract was then made in three concentrations, namely 50%, 75%, and 100%, with the addition of Vaseline and flava as a thickener for topical applications.

Gingivitis induction was carried out by installing a 3.0 silk ligature on the neck of the anterior teeth of the lower jaw of mice for six days to trigger accumulation.[16] Plaque and inflammatory response. After clinical signs of gingivitis, including erythema, edema, and gingival bleeding, developed, treatment was administered according to each group. Aloe vera extract was applied topically to the gingiva twice daily for five days. Gingival tissue was collected on days 3 and 5 after treatment for histopathological examination.

Tissue collection was performed by euthanizing the rats with a lethal dose of ketamine on days 3 and 5 after treatment, with six rats per group decapitated at each time point. Gingival tissue from the anterior region of the lower jaw was excised and fixed in 10% buffered formalin for 24 hours, followed by decalcification in 25% nitric acid for 24 hours. The specimens were sectioned, dehydrated through a series of graded ethanol solutions (70-100%), cleared in toluene and xylene, and embedded in paraffin blocks. Paraffin sections were cut using a microtome and stained with hematoxylin and eosin for histopathological examination.[14,15]

A histopathology observation sheet was used to count neutrophils and macrophages in hematoxylin-eosin-stained gingival tissue preparations. Observations were made using a light microscope at 40x magnification in five fields of view. This study was conducted in January 2025 at the Pharmaceutical Laboratory of Perintis Indonesia University and related histopathology laboratories in Padang City.

Data analysis was performed quantitatively. The inflammatory cell count data were tested for normality using the Kolmogorov–Smirnov test and for homogeneity using the Levene test. If the data met the assumptions of normality and homogeneity, the analysis was continued using a two-way ANOVA to determine the mean differences between groups and observation time. A Bonferroni post hoc test was performed if there were significant differences, with a significance level of $p<0.05$.

This research has obtained ethical approval and has been declared ethically sound by the authorized Health Research Ethics Commission, as stated in the Ethical Clearance Certificate No. A.007/KEPKFKGUNBRAH/XI/2024. This ethical clearance statement must be included in the scientific article manuscript as a form of compliance with the ethical principles of experimental animal research.

RESULTS AND DISCUSSIONS

Clinical observations showed that ligation of the anterior region of the lower jaw in rats for 6 days successfully triggered clinical signs of gingivitis, namely gingival swelling, reddish discoloration of the gingiva, and mild bleeding on exploration.

The gingival tissue of mice that had experienced gingivitis was removed by ligation thread, followed by treatment with a negative control in the form of Vaseline flava and aloe vera extract with concentrations of 50%, 75%, and 100% in different groups. This treatment was carried out twice daily for 5 days. Tissue sampling was carried out on the 3rd and 5th days after gingivitis induction for analysis using a gingivitis preparation. Histology, from the results of histological observations, the average number of neutrophil and macrophage cells was obtained as follows:

Table 1 Average number of neutrophil cells

Group	Day 3	Day 5
Negative control	1.67 cells/mm^3	1.67 cells/mm^3
Extract 50%	0.83 cells/mm^3	0.67 cells/mm^3
Extract 75%	0.67 cells/mm^3	0.17 cells/mm^3
Extract 100%	0.17 cells/mm^3	0 cells/mm^3

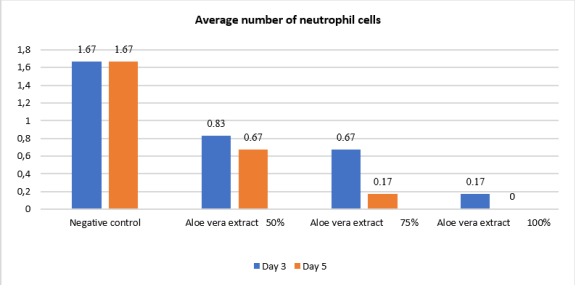

Figure 1. Average Number of Neutrophil Cells

Microscopic observations with 40x magnification shown in the table and graph of the average number of neurophil, it can be seen that the inflammatory cells decreased in the 50%, 75%, and 100% aloe vera extract groups from the 3rd to the 5th day with a significant decrease seen in the 100% concentration aloe vera extract treatment group, while in the negative control group the number of neutrophils did not decrease.

Table 2. Average number of macrophage cells

Group	Day 3	Day 5
Negative Control	2.5 cells/mm^3	2.5 cells/mm^3
Extract 50%	1.83 cells/mm^3	1 cells/mm^3
Extract 75%	1.17 cells/mm^3	0.3 cells/mm^3
100% Aloe Vera Extract	0.67 cells/mm^3	0 cells/mm^3

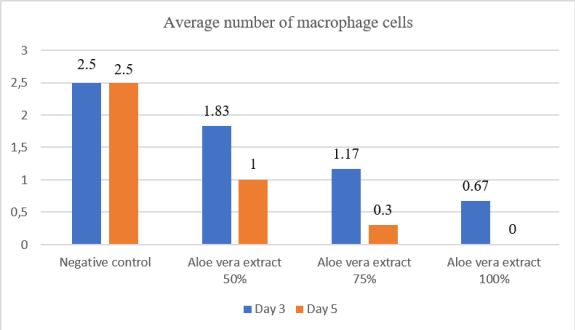

Figure 2. Average Number of Macrophage Cells

Microscopic observations with 40x magnification shown in the table and graph of the average number of macrophage cells, it can be seen that the inflammatory cells decreased in the 50%, 75%, and 100% aloe vera extract groups from the 3rd to the 5th day with a significant decrease seen in the 100% concentration aloe vera extract treatment group, while in the negative control group the number of macrophage cells did not decrease.

Clinical observations of the anterior mandibular gingiva of rats after the fifth day of treatment revealed that the gingiva was no longer swollen, its color returned to its original state, with the redness disappearing, and no bleeding was observed. This indicates that the gingivitis inflammation that occurred in the rats' gingiva had disappeared as a result of the treatment.

Normality test using *the Kolmogorov-Smirnov test* showed a significance value of 0.071 ($p > 0.05$), which indicates that the data is normally distributed. In addition, the results of the homogeneity test. The variance analysis using *Levene's test* yielded a significance value of 0.148 ($p > 0.05$), indicating that the data had homogeneous variance, and thus both assumptions for parametric analysis, namely normality and homogeneity, were met. The data can then be further analyzed using parametric tests. *Two-way ANOVA* to determine the effect of two independent variables on the dependent variable.

Table 3. *Two-Way ANOVA* test results for neutrophil cell count

Source	F	Sig
Treatment	19.215	0.000*
Time	2.816	0.101
Treatment-Time	1.743	0.174

Description: F: Calculated F value; *: ($p < 0.05$)

The Two-way ANOVA test in Table 3 shows a significant difference ($p < 0.05$) in the number of neutrophils between treatment groups. This indicates that the treatment given affects the number of neutrophil infiltration. Furthermore, to determine which groups have significant differences, a *Bonferroni post hoc test will be performed.*

Table 4. Summary of *Post Hoc* test results of *Bonferroni* neutrophil cell count

Treatment Group	P
Negative Control - 50%	0.004*
Negative Control - 75%	0.000*
Negative Control - 100%	0.000*
50%-75%	0.292
50%-100%	0.009*
75%-100%	1.000

Description: - : Interaction; * : Significant difference ($p < 0.05$)

The Bonferroni Post hoc test in Table 4 shows that the negative control group had a significant difference with the other three treatment groups, which was indicated by no decrease in the number of neutrophils. The 50% aloe vera extract treatment group did not show a significant decrease in neutrophil cells compared to the 75% treatment group, but there was a significant difference in the decrease compared to the 100% treatment group. Meanwhile, the 75% aloe vera extract treatment group did not show a significant difference in the decrease in neutrophils compared to the 100% treatment group. These results indicate that increasing the treatment concentration has a more pronounced effect up to a certain concentration.

Normality test using *the Kolmogorov-Smirnov test* showed a significance value of 0.200 ($p > 0.05$), which indicates that the data are normally distributed. In addition, the results of the homogeneity test and the variance analysis using *Levene's test* yielded a significance value of 0.270 ($p > 0.05$), indicating that the data had homogeneous variance, thus fulfilling both assumptions for parametric analysis, namely normality and homogeneity. The data can then be further analyzed using parametric

tests. *Two-Way ANOVA* to determine the effect of two independent variables on the dependent variable.

Table 5. Two-Way ANOVA test results for macrophage cell count

Source	F	Sig
Treatment	25.069	0.000*
Time	8.711	0.005*
Treatment-Time	1.151	0.340

Description: - : Interaction; F : Calculated F Value; * : (P < 0.05)

The two-way ANOVA test in Table 5 shows a significant difference (p < 0.05) in the number of macrophages between treatment groups and observation time. This indicates that the treatment and observation day given influence the number of macrophage cell infiltration. Furthermore, to determine which groups have significant differences, a *Bonferroni post hoc test will be performed.*

Table 6. Summary of *Bonferroni* macrophage cell count *Post Hoc* test results

Treatment group	p
Negative control - 50%	0.001*
Negative control - 75%	0.000*
Negative control - 100%	0.000*
50%-75%	0.084
50%-100%	0.002*
75%-100%	1.000

Description: -: Interaction; *: Significant difference (p < 0.05)

The Bonferroni Post hoc test in Table 6 shows that the negative control group had a significant difference with the other three treatment groups, which was indicated by no decrease in the number of macrophages. The 50% aloe vera extract treatment group did not show a significant decrease in macrophages compared to the 75% treatment group, but there was a significant difference in the decrease compared to the 100% treatment group. Meanwhile, the 75% aloe vera extract treatment group did not show a significant difference in the decrease in macrophages compared to the 100% treatment group. These results indicate that increasing the concentration per treatment provides a more significant effect up to a certain concentration.

The results of this study indicate that the administration of aloe vera extract (*Aloe barbadensis Miller*) reduces the number of inflammatory cells, namely neutrophils and macrophages, in the gingival tissue of mice with gingivitis. The decrease in the number of inflammatory cells is clearly visible in the treatment group compared to the negative control group, both on the 3rd and 5th day of observation. This indicates that aloe vera extract has anti-inflammatory activity that can control the inflammatory response in gingival tissue.

Analysis showed a decreasing trend in the number of neutrophils in the treatment group as the concentration of aloe vera extract increased. The most significant decrease was seen in the group given the extract at a concentration of 100%, especially on the 5th day of observation. This finding indicates that the anti-inflammatory effect of aloe vera is dose-dependent, with higher concentrations providing an inhibitory effect on inflammation. A decrease in the number of neutrophils reflects a reduced inflammatory response, which usually dominates the early phase of gingivitis.[3,5]

Similar results were also found in the number of macrophages. The treatment group showed a lower number of macrophages than the control group, especially on the 5th day after treatment. This decrease indicates that the continued inflammatory process and the release of pro-inflammatory mediators can be controlled by administering aloe vera extract. Macrophages are known to play a crucial role in maintaining chronic inflammation through their secretion of cytokines such as TNF-α and IL-1β, so a decrease in the number of these

cells is an important indicator of inflammation resolution. [4,6,17]

Biologically, the anti-inflammatory effects of aloe vera extract can be explained by its active compounds. Aloe vera contains flavonoids, saponins, tannins, polyphenols, and steroids, which are known to inhibit the cyclooxygenase and lipoxygenase pathways, reduce prostaglandin production, and inhibit thrombosis, migration, and activation of inflammatory cells. Furthermore, the active compounds in aloe vera also play a role in enhancing tissue regeneration and accelerating wound healing. [8,10]

The findings of this study align with previous research that reported that aloe vera has potential as a natural anti-inflammatory agent. Research by Sari et al. (2018) showed that administering aloe vera juice can reduce the number of leukocytes in mice experiencing inflammation.[12] Carolia and Sukohar[10] also reported that aloe vera extract with high concentrations was effective in reducing the number of macrophages in an inflammation model. oral mucosa.[10] In addition, Andri et al.[11] found that aloe vera extract can increase TGF-β levels, which play a role in the tissue healing process, thereby supporting the resolution of inflammation.[11]

Although the results of this study are consistent with previous research, this study provides complementary evidence by specifically examining the two main types of inflammatory cells (neutrophils and macrophages) simultaneously in a specific model of gingivitis, rather than presenting this dual assessment as a new technique. Since neutrophils and macrophages play roles in different phases of the inflammatory response, evaluating both simultaneously provides a more comprehensive picture of the anti-inflammatory mechanisms of aloe vera compared to evaluating only one cell type, as was done in most previous studies.

However, this study also has limitations. Observations were only conducted at two time points, on days 3 and 5, so the dynamics of inflammation in the early and later resolution phases cannot be fully described. Furthermore, this study did not evaluate the molecular expression of inflammatory mediators, such as proinflammatory cytokines, which can provide a deeper understanding of the mechanism of action of aloe vera extract.

Overall, the results of this study indicate that aloe vera extract is effective in reducing the number of neutrophils and macrophages in gingivitis, especially at high concentrations and over a longer observation period. These findings strengthen scientific evidence that aloe vera has the potential to be developed as a safe and effective natural alternative therapy for controlling gingival inflammation and can serve as a basis for further research in dentistry and periodontal health.

CONCLUSION

This study concluded that aloe vera (Aloe barbadensis Miller) extract reduced the number of neutrophils and macrophages in the gum tissue of Wistar rats with gingivitis, with the greatest effect observed at concentrations of 75% and 100% on day 5. These findings provide experimental evidence supporting the potential use of aloe vera as a natural anti-inflammatory agent for periodontal therapy.

REFERENCES

1. Tetan-el D, Adam AM, Jubhari, Hendra E. Gingival diseases: Plaque induced and non-plaque induced. Makassar Dental Jurnal. 2021;10(1):88-95. DOI: 10.35856/mdj.v10i1.394.
2. Safitri DN. Severity of gingivitis in pregnant women. Higeia Journal of Public Health Research and Development. 2020;4(3):470-479. DOI: 10.3390/jcm12093349.

3. Abdulkhaleq LA, Assi MA, Abdullah R, Saad MZ, Yap YH, Hezmee MN. The crucial roles of inflammatory mediators in inflammation: A review. Veterinary World. 2018;11(5):627-635. DOI: 10.14202/vetworld.2018.627-635.
4. Yuslianti ER, Heryawan, Koswara T, Pragita T. Effectiveness anti-inflammatory extract gambir leaf ethanol (Uncaria gambir.roxb) in reducing the number of inflammatory cells in Wistar rats with gingivitis: Experimental Laboratory. Journal of Dentistry Padjadjaran University. 2024;36(1):46-56. DOI:10.24198/jkg.v36i1.50120.
5. Rahmawati A, Pargaputri AF, S IK. The effect of giving brown algae extract of sargassum sp. on the number of macrophages in the healing process ulcer traumaticus. Denta Journal of Dentistry. 2018;12(1):72-81. DOI: 10.30649/denta.v12i1.163.
6. Puspaningrum EF, Hendari R, Mujayanto R. Extract Cymbopogon citratus and Eugenia aromaticum are effective for treating gingivitis. Odonto Dental Journal. 2015;2(2):47-51. DOI:10.30659/odj.2.2.47-51.
7. Fitri H, Fajrin FN, Kasuma N, Suharti, Netti. Effects of postpartum zinc administration scaling root planning on salivary MMP-8 Levels in gingivitis patients. B-Dent: Journal of Dentistry Baiturrahmah University. 2019;6(2):132-141. DOI:10.33854/jbd.v6i2.268.
8. Damanik DF, Monica Lubis YM, Meldawati. Antibacterial activity test of aloe vera extract against Pseudomonas aeruginosa and Streptococcus pyogenes. Andalas Medical Journal. 2021;44(6):357-364. DOI: 10.25077/mka.v44.i6.p357-364.2021.
9. Falsianingrum M, Retnaningsih A, Feladita N. Effectiveness test anti-inflammatory in preparation Aloe Vera Ointment (Aloevera L) for male rabbits (Oryctolagias cuniculus). Journal of Analysts Pharmacy. 2023;8(1):90-102. DOI:10.33024/jaf.v8i1.9915.
10. Carolia N, Sukohar A. The effect of giving aloe vera extract at concentrations of 25%, 50%, 75%, and 100% on the number of macrophages in inflammation oral mucosa of male white rats Sprague Dawley. JK Unila. 2016;1(2):243-246.
11. Andri NR, Elyani H, Andriana D. Comparison of the effects of aloe vera (Aloe Vera L) juice with 10% povidone iodine on serum transforming growth factor-beta (TGF-β) levels and fibroblast cell count in cut skin tissue on the backs of male Wistar rats. Journal of Bio Complementary Medicine. 2019;6(3):1-11.
12. Sari AR, Tjandrakirana, Kuswanti N. The effect of aloe vera juice on the number of leukocytes in White Rats (Rattus norvegicus) exposed to Cigarette Smoke. LenteraBio. 2018;7(2):159-162. Available from: http://ejournal.unesa.ac.id/index.php/lenterabio
13. Federer WT. Experimental Design: Theory and Application. New York: Macmillan; 1955.
14. Khristian E, Inderiati D. Sitohistoteknologi. Jakarta: Kementerian Kesehatan Republik Indonesia; 2017.
15. Apriani, Andrianus, Marisca S, Diana P. Ez Prep Concentrate (Ez Prep) sebagai alternative reagen deparafinisasi pada pewarnasaan hematoksilin eosin. G-Tech: Jurnal Teknologi Terapan. 2023;7(1):96-102. DOI:10.33379/gtech.v7i1.1874.
16. Rahman FA, Sunarintyas S, Martien R, Syaify A. Gingival inflammation induction in pregnant Sprague-Dawley rats: A pilot study. BIO Web of Conferences. 2023;75(2003):1-5. DOI:10.1051/bioconf/20237502003.
17. Ricardo AN, Oktariana D, Peranan makrofag dalam penyembuhan luka oral. Jurnal Kesehatan Gigi dan Mulut (JKGM). 2024;6(1):6-11. DOI:10.36086/jkgm.v6i1.2066.