

Anti-Inflammatory Effects of Pineapple Core Extract on Macrophages in Gingivitis- Induced Wistar Rats

by Perpustakaan IIK Bhakti Wiyata

Submission date: 23-Apr-2026 05:00PM (UTC+0700)

Submission ID: 2501079557

File name: 19643-62025-1-PB_-_ANGGRAINI_DYAH_SETIYARINI.pdf (436.5K)

Word count: 3858

Character count: 23544

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Anti-Inflammatory Effects of Pineapple Core Extract on Macrophages in Gingivitis-Induced Wistar Rats

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ARTICLE INFO

Keywords:

Pineapple Core Extract; Bromelain; Macrophage; Gingivitis; Wistar Rats

Article History:

Received : 23/10/2025

Revision : 05/02/2026

Accepted : 13/02/2026

Published : 16/02/2026

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ISSN: 2775-0159



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ABSTRACT

Background: Gingivitis is the earliest stage of periodontal disease, characterized by gingival inflammation, edema, and bleeding. This condition involves infiltration of inflammatory cells, including macrophages, which play a regulatory role through pathogen phagocytosis and pro-inflammatory cytokine secretion. Pineapple core (*Ananas comosus* L. Merr) contains several bioactive constituents—such as bromelain, flavonoids, and tannins—that have been reported in the literature to exhibit anti-inflammatory and antioxidant properties. Bromelain, a proteolytic enzyme abundantly present in pineapple core according to previous studies, is known to modulate inflammatory pathways through cytokine suppression.

Objective: This study aimed to evaluate the effect of pineapple core extract on macrophage cell counts in gingivitis-induced Wistar rats.

Method: A post-test only design was used with 30 male rats allocated into three groups: control (CMC-Na), 12.5% extract, and 25% extract. Gingivitis was induced, followed by three days of treatment. Gingival tissues were processed histologically, and macrophage-like cells were quantified under light microscopy.

Outcome: The mean macrophage counts were 27.38 (control), 13.60 (12.5%), and 7.66 (25%), with significant differences among groups ($p < 0.05$).

Conclusion: These findings indicate that pineapple core extract can reduce macrophage infiltration in gingival tissues, and the higher concentration demonstrates a stronger anti-inflammatory effect.

Citation (Vancouver style):

Chasanah N, Pertami SDI, Rahmawan D, Anwar R, Agustina GD. Anti-inflammatory effects of pineapple core extract on macrophages in gingivitis-induced Wistar rats. *Indones J Dent.* 2026;6(1): 63-72.

INTRODUCTION

Periodontal diseases represent a major oral health concern across the globe and continue to pose a substantial public health burden. Gingivitis is recognized as the earliest and most prevalent stage of periodontal conditions, and without proper management it may advance into more destructive forms such as periodontitis. According to the Indonesian Basic Health Research (Riskasdas 2018), more than 70% of the population across various age groups is affected by periodontal disease, indicating its significant contribution to the national oral health burden.¹ Clinically, gingivitis is marked by gingival redness, swelling, bleeding, and discomfort, which reflect an underlying inflammatory reaction.² If this inflammatory process persists, it may eventually cause attachment loss and tooth loss, thereby reducing quality of life and increasing the need for dental treatment. These considerations highlight the importance of effective preventive and therapeutic strategies aimed at controlling gingival inflammation.

¹⁶ The development of gingivitis is closely linked to the host's immune reaction toward dental plaque biofilm. The accumulation of bacteria triggers the recruitment of various immune cells—including neutrophils, lymphocytes, and macrophages—into the gingival tissues. Macrophages function both as phagocytic cells and as antigen-presenting cells, and they contribute significantly to the maintenance of inflammation by releasing multiple pro-inflammatory mediators. These include cytokines ⁵ such as TNF- α and IL-1 β , reactive oxygen metabolites, and enzymes like COX-2, all of which amplify the inflammatory cascade.^{3,4} Because of their major regulatory role in inflammation, macrophage numbers are commonly utilized as markers to assess gingival inflammatory status and healing progression.⁵ In line with this, increased inflammatory cell infiltration—especially macrophages—has been consistently reported in gingival tissues affected by gingivitis and periodontitis, and tends to decline following supportive herbal-based therapeutic interventions.⁶

Management of gingivitis generally focuses on controlling dental plaque and ⁴ the use of non-steroidal anti-inflammatory drugs (NSAIDs). Although these medications can effectively suppress inflammatory responses, extended use often leads to gastrointestinal complications, which limits their suitability for long-term periodontal treatment.⁷ Because of these drawbacks, there is growing interest in exploring safer therapeutic options derived from natural products. Recent studies indicate that herbal formulations possess anti-inflammatory, antioxidant, and tissue-repair-enhancing properties that may aid periodontal healing and function as adjunctive approaches in gingivitis therapy.⁸

Pineapple (*Ananas comosus* L. Merr) is a tropical plant known for its various medicinal benefits. The honey pineapple variety grown in Kediri Regency, Indonesia, is reported to contain substantial amounts of bromelain in its core, along with other phytochemicals such as flavonoids and tannins.⁹ Bromelain is a proteolytic enzyme recognized for its anti-inflammatory, antioxidant, and immunomodulatory properties, and it can influence cytokine production as well as the movement of inflammatory cells.^{9,10} Previous investigations further demonstrated that bromelain can downregulate central inflammatory mediators—including IL-1 β , IL-6, and TNF- α —which contributes to a decrease in inflammatory cell infiltration in periodontal tissues.^{10,11} Although bromelain is widely acknowledged as the main bioactive compound of pineapple core, its exact concentration in the extract used in this study was not quantified. Nonetheless, the natural presence of bromelain and additional phytochemicals in pineapple core suggests that the extract may support the resolution of gingival inflammation by modulating macrophage activity. Based on this rationale, the present study evaluated the anti-inflammatory effects of pineapple core extract on macrophage cells in gingivitis-induced Wistar rats.

RESEARCH METHODS

This research employed an in vivo experimental laboratory approach with a post-test-only control group format. Thirty healthy male Wistar rats (*Rattus norvegicus*), aged approximately 2–3 months and weighing 150–200 g, were used in this study. The animals were sourced from the Laboratory of Experimental Animals, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya. Prior to the intervention, all rats were acclimatized under controlled laboratory settings, including a 12-hour light/dark cycle, regulated temperature, and provision of a standard pellet diet with unrestricted access to drinking water. Ethical approval for all procedures was granted by the Health Research Ethics Committee of the Faculty of Dentistry, Institut Ilmu Kesehatan Bhakti Wiyata, Kediri (Approval No. 22/FKG/EP/III/2022). All experimental stages adhered to institutional animal care and welfare guidelines.

Fresh honey pineapple (*Ananas comosus* L. Merr) cores were sourced from Kediri Regency, East Java, Indonesia. After collection, the cores were cleaned, dried, and macerated in 96% ethanol for 72 hours, followed by filtration and concentration under reduced pressure using a rotary evaporator to produce a semi-solid extract. This concentrated extract was subsequently mixed with carboxymethylcellulose sodium (CMC-Na) to obtain 12.5% and 25% preparation strengths. Bromelain is recognized in prior studies as a principal bioactive component of pineapple core, supported by flavonoids and tannins that also contribute to its

anti-inflammatory properties; however, the exact bromelain content in the extract used in this study was not quantified.

¹⁸ The animals were assigned to three experimental groups of ten rats each, with Group 1 serving as the negative control and receiving CMC-Na gel, while Groups 2 and 3 ¹ were treated with pineapple core extract at concentrations of 12.5% and 25%, respectively. Gingivitis was induced by tying a 3.0 silk ligature around the cervical region of the mandibular incisors on both sides, and the ligatures were left in place for three days to provoke inflammation through plaque retention and mechanical irritation, a method commonly applied in periodontal inflammation studies.¹¹ After the induction period, the designated topical preparations were applied once daily for three days based on each group's treatment assignment.

On the fourth day, all animals were euthanized under ketamine anesthesia. Gingival tissue from the anterior mandibular region was excised and immediately placed in 10% buffered formalin for a 24-hour fixation period. The samples were then processed through dehydration, paraffin embedding, and trimmed into 5- μ m sections using a microtome. These ¹² tissue slices were subsequently stained with hematoxylin and eosin (H&E) for microscopic evaluation. Macrophage identification was based on established morphological features, including a relatively large cell size, a foamy eosinophilic cytoplasm, an eccentrically positioned oval-to-kidney-shaped nucleus, and irregular cellular borders. Cells that met these criteria were counted at 400 \times magnification across five randomly selected high-power fields (HPFs) for each specimen.¹²

The dataset was organized and processed using IBM SPSS Statistics version 25.0. Assessment of data distribution was carried out with the Shapiro-Wilk test, and variance homogeneity was verified using Levene's test. Differences in mean macrophage counts among the experimental groups were evaluated through a one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) procedure for post hoc pairwise comparisons. A p-value of less than 0.05 was considered statistically significant. ²

RESEARCH FINDINGS

The evaluation of macrophage cell infiltration was carried out after three days of treatment in all experimental groups. Gingival tissues from the negative control group (CMC-Na) showed the highest mean macrophage cell count of 27.38 cells/HPF. Each mean value represents observations from 10 animals per group and is presented as mean \pm standard deviation (SD) to reflect data variability. This finding indicates that gingival inflammation

induced by ligature placement triggered a strong inflammatory response characterized by the accumulation of macrophages in the gingival tissue. In contrast, the groups treated with bromelain-containing pineapple core extract exhibited a noticeable reduction in macrophage cell count, reflecting the anti-inflammatory effect of the extract.

In the treatment group receiving pineapple core extract at a concentration of 12.5%, the mean macrophage cell count decreased to 13.60 cells/HPF. This represents almost a 50% reduction compared with the negative control group. Histologically, gingival tissues in this group still showed inflammatory cell infiltration, but the density of macrophages was markedly lower than in the control group. This finding suggests that even at a lower concentration, bromelain-containing pineapple core extract is capable of attenuating gingival inflammation by limiting macrophage recruitment.

The most pronounced effect was observed in the group treated with pineapple core extract at 25%, where the mean macrophage cell count was only 7.66 cells/HPF. This reduction corresponds to approximately a 72% decrease compared with the control group. Gingival histology in this group demonstrated fewer inflammatory cells, with tissue structure beginning to return closer to normal. These results indicate that the higher concentration of pineapple core extract is more effective in suppressing macrophage infiltration and may accelerate the resolution of inflammation in gingivitis.

Statistical analysis supported these findings. Assessment of data distribution using the Shapiro-Wilk test and evaluation of variance uniformity through Levene's test showed that the dataset met the assumptions required for parametric testing. Subsequently, One-Way ANOVA demonstrated highly significant differences across the three experimental groups, indicated by an F-value of 484.610 and a p-value of 0.000 (Table 2). These outcomes confirm that administering pineapple core extract produced a meaningful and statistically significant reduction in macrophage counts within gingival tissues.

Further evaluation using Tukey's HSD post hoc test (Table 3) showed that every pairwise comparison between groups yielded statistically significant differences ($p < 0.05$). The contrast between the control group and the 12.5% extract group indicated a notable decrease in macrophage numbers, while the comparison between the 12.5% and 25% extract groups demonstrated that the higher dose produced a stronger anti-inflammatory response. Similarly, the comparison involving the control group and the 25% extract group revealed the most substantial reduction. Overall, these outcomes confirm that pineapple core extract at both tested

concentrations effectively lowers macrophage infiltration, with the 25% formulation producing the greatest anti-inflammatory impact.

Table 1. Mean Macrophage Cell Count in Gingival Tissues of Wistar Rats

Group	Treatment	Mean (cells/HPF)
1	Negative control (CMC-Na)	27,38
2	Pineapple core extract 12.5%	13,6
3	Pineapple core extract 25%	7,66

Table 2. The Table Summarizes the One-Way ANOVA Findings for Macrophage Count Averages.

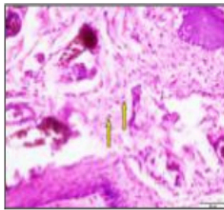
Source of Variation	Sum of Squares	df	Mean Square	F	Sig. (p)
Between Groups	1778675	2	889338		
Within Groups	45879	25	1835	484610	0.000 *
Total	1824554	27			

All values are reported as mean with their corresponding standard deviation, based on 10 rats per experimental group. Analysis using one-way ANOVA showed that the groups differed significantly ($p < 0.05$).

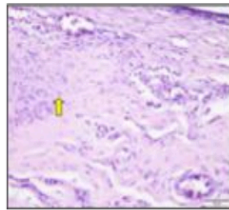
Table 3. Tukey's HSD Post Hoc Procedure was Applied for Comparisons Across Groups

Group	N	Subset for $\alpha = 0.05$	Mean (cells/HPF)
25%	10	1	7,66
12.5%	10	2	13,6
Control	10	3	27,38

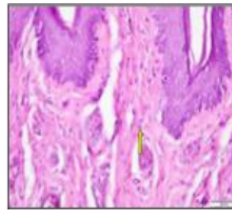
Results summarized as mean \pm SD (n = 10 per group). All pairwise contrasts reached statistical significance ($p < 0.05$). Analysis using Tukey's HSD post hoc procedure indicated that all pairwise group comparisons were statistically significant ($p < 0.05$).



I.1 Tissue Microscopic of the negative control group



I.2 Tissue Microscopic of the 12.5% treatment group



I.3 Tissue Microscopic of the 25% treatment group

Figure 1. Histological features of gingival tissue processed with hematoxylin–eosin (H&E) staining.

Yellow arrows highlight macrophage-like cells identified by their typical morphology, including enlarged cell bodies, eosinophilic and foamy cytoplasm, and an eccentric oval-to-kidney-shaped nucleus. Specifically, (1.1) the negative control group (CMC-Na) showed dense

inflammatory infiltration; (1.2) the treatment group with 12.5% pineapple core extract showed reduced macrophage density; and (1.3) the treatment group with 25% pineapple core extract showed the lowest inflammatory cell infiltration.

DISCUSSION

The present study demonstrated that pineapple core extract significantly reduced macrophage infiltration in gingival tissues of Wistar rats with ligature-induced gingivitis. The negative control group treated with CMC-Na exhibited the highest mean macrophage count (27.38 cells/HPF), reflecting the inflammatory response triggered by plaque accumulation and mechanical irritation from ligatures.¹¹ In contrast, the groups treated with pineapple core extract at 12.5% and 25% concentrations showed markedly lower macrophage counts, with the 25% concentration producing the most substantial reduction (7.66 cells/HPF). These findings indicate a concentration-dependent anti-inflammatory effect of the pineapple core extract in attenuating inflammatory cell recruitment. Hematoxylin and eosin staining for macrophages is indeed a non-specific method; however, it remains acceptable for preliminary quantification in rodent gingivitis models when macrophage-specific morphological features—such as large cell size, abundant eosinophilic cytoplasm, an eccentric oval-to-kidney-shaped nucleus, and irregular cell borders—are clearly defined.¹² Several experimental studies in periodontal and gingival wound-healing models have similarly used hematoxylin and eosin-based quantification to assess inflammatory cell infiltration when immunohistochemical facilities were unavailable, supporting the methodological validity of the present study.⁵ Consistent with these findings, previous investigations involving natural-product-based interventions have also reported significant reductions in inflammatory cell infiltration in experimental gingivitis and periodontitis models, further reinforcing the therapeutic potential of botanical compounds in periodontal disease management.¹⁵

Macrophages are central contributors to the development of gingivitis because they function both as phagocytic cells and as key regulators of inflammatory activity. These cells release mediators such as TNF- α , IL-1 β , reactive oxygen species (ROS), and COX-2, all of which intensify the inflammatory cascade and attract additional immune cells to the gingival tissues.^{3,4} The elevated macrophage infiltration observed in the control group aligns with earlier findings demonstrating that gingivitis and periodontitis tissues contain markedly higher macrophage numbers compared with healthy gingiva.^{6,11} Furthermore, recent literature highlights that the shift of macrophages from the M1 (pro-inflammatory) phenotype toward the M2 (anti-inflammatory) phenotype represents an essential component of periodontal tissue

repair.¹⁶ The notable reduction in macrophage counts found in this study suggests that pineapple core extract may downregulate macrophage recruitment and potentially influence polarization mechanisms, thereby aiding the resolution of gingival inflammation.

The anti-inflammatory properties of bromelain have been widely recognized in previous research. This enzyme modulates inflammation by influencing several intracellular signaling mechanisms, including the nuclear factor kappa B (NF- κ B) pathway and mitogen-activated protein kinases (MAPKs), both of which play important roles in regulating pro-inflammatory cytokine synthesis.⁹ Insuan et al. found that bromelain markedly reduced the production of TNF- α , IL-1 β , and IL-6 in macrophages by inhibiting NF- κ B and MAPK activation.⁹ Likewise, Kasemsuk and colleagues reported that bromelain diminished cytokine and chemokine secretion in macrophages stimulated with lipopolysaccharide.¹⁰ These documented mechanisms provide a plausible explanation for the decrease in macrophage infiltration observed in this study. Although bromelain content within the extract was not quantified, the anti-inflammatory effects identified suggest that the phytochemical constituents present in pineapple core extract may act through molecular pathways typically attributed to bromelain.

These results align with findings from earlier animal experiments showing that several plant-derived preparations—including *Moringa oleifera* and green tea catechins—can lessen inflammatory cell presence within gingival tissues.^{8,20} Prior research has also identified bromelain as a key bioactive compound in pineapple core with notable anti-inflammatory properties. Evidence indicates that bromelain may modulate macrophage behavior by affecting cytokine secretion and cell-infiltration pathways,⁹ and that both the pineapple stem and core are recognized as botanical sources containing relatively high amounts of this enzyme.^{13,14} Moreover, bromelain has been described as possessing a wide range of biological actions—such as anti-inflammatory, antioxidant, antiedematous, and wound-healing effects—according to recent pharmacological literature.¹⁷ Although this study did not measure bromelain concentration directly or compare its performance with other phytotherapeutic agents, the mechanisms previously documented offer a reasonable explanation for the anti-inflammatory response observed with the pineapple core extract in this investigation.

Conventional therapy for gingivitis primarily involves mechanical plaque removal and the use of non-steroidal anti-inflammatory drugs (NSAIDs). Although these medications can effectively reduce inflammatory responses, extended administration is frequently linked to gastrointestinal complications such as ulceration and bleeding.⁷ Findings from recent investigations on herbal adjuncts^{18,19} indicate that pineapple core extract—which contains

bromelain and various phytochemicals—may serve as an additional therapeutic option with a potentially better safety margin. Although this study did not evaluate bromelain concentration or conduct a safety assessment, the anti-inflammatory response observed suggests that pineapple core extract could be considered as a supportive agent in gingivitis management, especially in communities with limited access to advanced periodontal care.

The clinical implications of these findings are noteworthy. A reduction in macrophage infiltration suggests that pineapple core extract may help regulate gingival inflammation—an early pathological phase in periodontal disease during which macrophage activation contributes to tissue damage and eventual bone loss.^{2,6} The combination of several bioactive components, including bromelain, flavonoids, and tannins, indicates that the extract may exert anti-inflammatory effects through multiple mechanisms. Recent clinical investigations involving herbal adjuncts—such as curcumin-based gels and green tea catechins—have shown improvements in gingival parameters and inflammatory biomarkers.²⁰ Although the current study did not measure bromelain concentration or evaluate periodontal outcomes directly, the anti-inflammatory pattern observed supports the potential for pineapple core extract to be further investigated as a complementary agent in periodontal therapy.

Several limitations should be noted. This study used an animal model with a short, three-day treatment period, which reflects only acute inflammatory responses and may not fully represent clinical conditions in humans. Additionally, the extract was not analyzed for its bromelain content limiting the ability to directly correlate phytochemical concentration with biological effects.

CONCLUSION

This study demonstrated that pineapple core extract effectively reduced macrophage infiltration in the gingival tissues of Wistar rats with ligature-induced gingivitis. The reduction was concentration-dependent, with the 25% extract producing the most pronounced anti-inflammatory effect compared with the 12.5% and control groups. Although prior literature reports bromelain as a major constituent of pineapple core, its level was not quantified in this study.

ACKNOWLEDGMENTS

The authors express the gratitude to the Faculty of Dentistry, Institut Ilmu Kesehatan Bhakti Wiyata Kediri, for providing research facilities, and to the Faculty of Dentistry, Universitas Muhammadiyah Semarang, for their valuable academic input.

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