ORIGINAL ARTICLE

Effectiveness of rambutan honey toothpaste on IL-6 gene expression during acute inflammatory phase of gingivitis in a Wistar rat model: an experimental study

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ABSTRACT

Introduction: Gingivitis is a reversible form of periodontal disease characterized by gingival inflammation due to dental plaque accumulation and increased inflammatory mediators such as IL-6. Rambutan honey (*Nephelium lappaceum*) contains bioactive compounds with potential as natural anti-inflammatory agents. This study aimed to analyze the effectiveness of rambutan honey toothpaste on IL-6 gene expression during acute inflammatory phase of gingivitis in a Wistar rat model. **Method:** This study used a true experimental design with a post-test only control group design. A total of 30 male Wistar rats were randomly divided into three groups: negative control (toothpaste base/NC), positive control (commercial herbal toothpaste/PC), and treatment (rambutan honey/TPRH). The gingivitis rat model was established using a ligature technique with silk thread and inoculation of *Porphyromonas gingivalis* and *Streptococcus mutans*. Treatment was administered twice daily for seven days, and gene expression was evaluated on days 0, 3, 5, and 7 using qRT-PCR. Data were analyzed using ANOVA and post hoc Tukey tests ($p<0.05$). **Result:** The rambutan honey toothpaste group showed a decreasing pattern of IL-6 expression compared to the base and commercial toothpaste groups, particularly on day 3 ($p=0.038$). These findings suggest that rambutan honey toothpaste may reduce IL-6 expression. This effect may be related to the presence of flavonoid and phenolic compounds in rambutan honey, which have been associated with anti-inflammatory activity. **Conclusion:** Rambutan honey toothpaste was associated with reduced IL-6 gene expression during the early acute inflammatory phase of experimental gingivitis in Wistar rats, indicating a potential anti-inflammatory effect at the molecular level. Further studies, including clinical, histological, and protein-level assessments, are needed to confirm its relevance in gingival healing.

KEYWORDS

Gingivitis, honey, interleukin-6, toothpaste, wistar rat

INTRODUCTION

Gingivitis is a common inflammatory condition of the gingiva caused by bacterial plaque accumulation, and if left untreated, it may progress to periodontitis, leading to irreversible damage of the supporting periodontal structures.[1,2] Epidemiological data indicate that gingivitis remains highly prevalent worldwide and continues to pose a significant public health burden. In Indonesia,

the 2018 Basic Health Research (Riskesdas) reported a gingivitis prevalence of 15.8% in individuals aged 25–34 years and 16.6% in those aged 35–44 years, with a slightly higher occurrence in females than males.[3] Beyond local clinical manifestations such as gingival bleeding, swelling, and pain, gingivitis negatively affects oral health–related quality of life (OHRQoL) and has been associated with systemic conditions, including diabetes mellitus and coronary heart disease.[4–6]

The inflammatory response in gingivitis is primarily driven by host–microbial interactions, with proinflammatory cytokines playing a pivotal role in disease initiation and progression. Interleukin-6 (IL-6) is a key mediator of gingival inflammation that regulates immune responses, stimulates hepatic C-reactive protein production, and contributes to connective tissue degradation.[7] Increased IL-6 expression has been consistently reported in gingivitis and periodontitis and is positively correlated with disease severity and delayed periodontal healing.[8] Elevated IL-6 not only reflects local inflammation but also links periodontal disease with systemic disorders such as diabetes and cardiovascular disease.[6,9] These findings support the use of IL-6 as a reliable biomarker for evaluating gingival inflammation and therapeutic efficacy.

Conventional gingivitis management relies on mechanical plaque control supported by chemical agents incorporated into toothpaste formulations. However, prolonged use of synthetic antimicrobial and anti-inflammatory agents may be associated with adverse effects, disruption of the oral microbiota, and reduced patient compliance. Consequently, natural products with anti-inflammatory and antioxidant properties, such as rambutan honey, are gaining increasing interest as alternative or adjunctive therapies in oral healthcare.[8,10] Honey contains flavonoids, phenolic acids, vitamin C, and antioxidant enzymes that inhibit NF-κB activation and reduce IL-6 expression.[11-13]

Previous experimental studies on rambutan honey have demonstrated its ability to reduce oxidative stress, accelerate mucosal wound healing, and inhibit oral pathogens such as *Streptococcus* species.[10] However, most studies remain in vitro, and evidence regarding its application in toothpaste formulations for in vivo gingivitis models is still limited.[10] In addition, the temporal pattern of inflammatory marker suppression during the early phase of gingival healing has not been adequately explored. The novelty of this research lies in the evaluation of a rambutan honey–based toothpaste using a ligature- and bacteria-induced gingivitis rat model, with sequential assessment of IL-6 gene expression over multiple observation time points.[11-13]

Using the PICOS framework, the participants were Wistar rats with experimentally induced gingivitis (P), the intervention was toothbrushing with rambutan honey toothpaste (I), the comparisons were toothpaste base and a commercial herbal toothpaste (C), the outcome was relative IL-6 gene expression in gingival tissue (O), and the study design was a true experimental post-test only control group design (S). We hypothesized that rambutan honey toothpaste would suppress IL-6 expression more rapidly than control formulations, thereby accelerating the resolution of gingival inflammation.[14–15]

The novelty of this research lies in providing evidence regarding the in vivo anti-inflammatory effect of rambutan honey–based toothpaste during the acute phase of gingivitis, particularly at the molecular level. Furthermore, changes in IL-6 gene expression following topical toothpaste application have not been adequately investigated. Therefore, this study aims to evaluate the anti-inflammatory effect of rambutan honey toothpaste on gingival healing by analyzing IL-6 gene expression in gingivitis-induced Wistar rats.

METHODS

This study was a true experimental research with a post-test only control group design. All experimental procedures were conducted in accordance with the

ARRIVE guidelines and international standards for animal research. Male Wistar rats (*Rattus norvegicus*), aged 6–8 weeks and weighing 150–250 g, were used in this study. Animals were housed under standard laboratory conditions (12 h light/dark cycle, temperature 22–25 °C, relative humidity 50–60%) with ad libitum access to standard chow and water. Inclusion criteria included healthy appearance, normal activity, and absence of anatomical abnormalities, while exclusion criteria were illness during acclimatization, mortality before intervention, or body weight loss exceeding 20%.

Sample size determination followed Federer's formula $(5(n-1)(t-1) \geq 15)$, where n represents the number of experimental animals per group and t represents the number of experimental groups. In this study, three groups were included (t=3): negative control (toothpaste base), positive control (commercial herbal toothpaste/Pepsodent Herbal), and treatment group (rambutan honey toothpaste). Substituting t=3 into the formula yields $5(n-1)(2) \geq 15$, resulting in $n \geq 9$ animals per group. To account for potential attrition during the experimental period, ten rats were allocated to each group, giving a total of 30 animals.

Rats were randomly assigned to the three groups using a computer-generated randomization sequence. Allocation concealment was ensured by labeling toothpaste containers with coded identifiers that were unknown to the investigators performing treatment administration and outcome assessment.

The tools used in this study included standard animal handling equipment, sterile gloves, fine scissors, forceps, oral applicator brushes, micropipettes with sterile RNase-free tips, a centrifuge, vortex mixer, biosafety cabinet, incubator, freezer (−20°C and −80°C), spectrophotometer (Multiskan™ GO), and a qRT-PCR system (AriaMx, Agilent).[8]

The rambutan honey toothpaste was formulated at the Faculty of Pharmacy, Universitas Jenderal Achmad Yani, using pharmaceutical-grade rambutan honey. The formulation consisted of rambutan honey (20% w/w), calcium carbonate (35% w/w), glycerin (20% w/w), sodium carboxymethylcellulose (1.5% w/w), sodium lauryl sulfate (1% w/w), sodium benzoate (0.2% w/w), saccharin (0.1% w/w), menthol (0.3% w/w), and distilled water q.s. to 100%. All ingredients were mixed under standardized conditions to ensure batch-to-batch reproducibility. Each brushing session used approximately 50 mg of toothpaste per rat, applied using a small applicator brush. Using body surface area normalization methods, this dose approximates a clinically relevant human toothpaste exposure. Toothbrushing was performed twice daily for seven consecutive days.[8]

Treatment administration and outcome assessment were performed by investigators blinded to group allocation, while a separate researcher maintained the randomization code until data analysis was completed. After a seven-day acclimatization period, experimental gingivitis was induced following a standardized ligature- and bacteria-induced model described in previous studies. Briefly, animals were anesthetized using intraperitoneal ketamine (80 mg/kg) and xylazine (10 mg/kg) prior to the procedure. A sterile 3-0 nylon ligature was placed around the lower incisors at the gingival margin to promote plaque accumulation.

Subsequently, a bacterial suspension containing *Porphyromonas gingivalis* (ATCC 33277) and Streptococcus mutans (ATCC 25175), each at a concentration of 1×10^9 CFU/mL, was prepared in phosphate-buffered saline. A total volume of 0.1 mL of the mixed bacterial suspension was topically inoculated into the gingival sulcus once daily for five consecutive days, resulting in a multiplicity of infection (MOI) of approximately 100 bacteria per host cell. No antibiotics or special diets were administered during the experiment. Gingivitis induction was confirmed clinically by the presence of gingival erythema, edema, and bleeding on gentle probing, which typically developed within 6–7 days after ligature placement (Figure 1).[16,17]

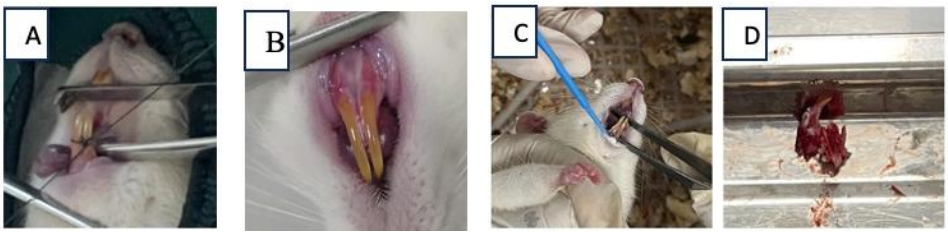

Figure 1. Experimental procedures in the gingivitis rat model. A, ligature placement on lower incisors. B, clinical appearance of gingivitis after bacterial induction. C, application of toothpaste using an applicator brush. D, excised gingival tissue for molecular analysis.

Treatment was administered by brushing the respective toothpaste onto the labial surface of the rat lower incisors twice daily (morning and evening) for seven consecutive days using a small sterile applicator brush. Following confirmation of gingivitis induction, animals were randomly allocated into three groups: (1) negative control (NC), receiving placebo toothpaste; (2) positive control (PC), receiving a commercial herbal toothpaste; and (3) treatment group (TPRH), receiving rambutan honey–based toothpaste.

Gingival tissue samples were collected sequentially at predefined observation time points, namely day 1, day 3, and day 7 after initiation of toothpaste treatment. The timing of tissue collection was designed to capture the early, intermediate, and late phases of gingival inflammatory resolution. Gingival tissues from rats were finely minced, and RNA was isolated and purified from each experimental group using the Direct-zol RNA Miniprep Plus Kit (Zymo, R2073) following the manufacturer's protocol. Complementary DNA synthesis was carried out with the iScript Reverse Transcription Supermix for RT-PCR (Bio-Rad, 170 8841). Gene expression levels were quantitatively analyzed using the Agilent AriaMx 3000 real-time PCR system with Evagreen Master Mix (Bio-Rad, 1725200).[8]

Total RNA was extracted from gingival tissues (~30 mg) using TRI Reagent® (Zymo Research, R2050-1-200) combined with Direct-zol™ RNA Miniprep Plus Kit (Zymo Research, R2073). Tissues were homogenized in TRI Reagent with a grinder, followed by ethanol addition and purification through spin columns. RNA was washed with kit-provided buffers and eluted with RNase-free water. RNA concentration and purity were measured with Multiskan™ GO spectrophotometer, and samples with A260/A280 ratios between 1.8 and 2.0 were considered pure.

RNA was reverse-transcribed into cDNA using the SensiFAST™ cDNA Synthesis Kit (Meridian Bioscience, BIO-65054). The procedure consisted of priming at 25°C for 5 min, reverse transcription at 46°C for 20 min, and enzyme inactivation at 95°C for 1 min.[8] Gene expression was analyzed using qRT-PCR with the AriaMx Real-Time PCR System (Agilent, G8830A) and SYBR Green Master Mix. Specific primers for IL-6 and the housekeeping gene (GAPDH) were used for amplification. The comparative Ct (ΔΔCt) method was applied to evaluate relative gene expression across groups. All samples were run in duplicates or triplicates for accuracy.[8]

Statistical analysis was performed using the Statistical Program for Social Science (SPSS). Data were assessed for normality and homogeneity. Levene's test was used to assess homogeneity, while the Shapiro-Wilk test was applied to evaluate normality since the sample size is less than 50. If the data were normally distributed, analysis proceeded with a One-Way ANOVA at a 95% confidence level ($\alpha = 0.05$). If the data did not follow a normal distribution, the Kruskal-Wallis test was applied for variance analysis. Subsequently, Post Hoc Tukey analysis was performed.

RESULTS

RNA concentration and purity were assessed spectrophotometrically by measuring absorbance at 260 and 280 nm. The results demonstrated that all samples met the acceptable criteria for RNA quality, with A260/A280 ratios indicating adequate purity for downstream qRT-PCR analysis. The results of RNA purity and concentration obtained from representative samples of each treatment group at different observation days (day 0 to day 7) are presented in Table 1.

Table 1. RNA purity and concentration (mean values) of representative samples from each treatment group from day 0 to day 7

Sample	Days	Concentration (ng/µl)	Purity (λ260/λ280nm)
NC	0	39.6400	2.0635
	1	12.6800	2.0725
	3	25.1200	2.1184
	5	70.5600	2.0779
	7	26.4400	2.3498
PC	0	27.8000	2.0904
	1	58.3600	1.9876
	3	40.7600	1.9681
	5	10.3200	1.4583
	7	35.2000	1.8977
TPRH	0	65.3600	1.7439
	1	26.9600	2.2543
	3	28.1600	2.1958
	5	10.1600	2.0664
	7	28.8800	1.9917

NC: Negative Control (Gingivitis rats + toothpaste base 2x/day), PC: Positive Control (Gingivitis rats + commercial toothpaste 2x/day), TPRH (Gingivitis rats + Rambutan Honey toothpaste 2x/day).

The primer sequences used for quantitative real-time PCR analysis, including both target and reference genes, were synthesized commercially (Macrogen, South Korea) and are summarized in Table 2.

Table 2. Primer sequences used in qRT-PCR analysis

Gene	Primer Sequences (5′–3″)	Product Length (bp)	Annealing Temperature (°C)
IL-6 (*Rattus norvegicus*)	F: TGATGGATGCTTCCAAACTG R: GAGCATTGGAAGTTGGGGTA	230	53

The IL-6 values presented in Table 3 represent relative gene expression levels calculated using the $2^{-\Delta\Delta Ct}$ method. The data are expressed as fold change relative to the negative control at Day 0. No conversion to absolute concentration (pg/mL) was performed, as protein quantification by ELISA was not conducted in this study.

Table 3. Relative IL-6 gene expression (fold change, $2^{-\Delta\Delta Ct}$) in gingival tissue across treatment groups and observation days

Observation Day	Group			P-value
	NC (Fold change)	PC (Fold change)	TPRH (Fold change)	
Day 0	4.8761a ± 0.65	4.8242a ± 0.12	4.9852a ± 0.22	0.886
Day 1	4.7317a ± 0.33	4.3272a ± 0.37	4.8183a ± 0.49	0.347
Day 3	4.1698b ± 0.91	3.1078a ± 0.34	3.4360ab ± 0.13	0.038*
Day 5	1.0043a ± 0.11	0.9364a ± 0.16	0.9592a ± 0.08	0.794
Day 7	0.8171a ± 0.05	0.7762a ± 0.07	0.8411a ± 0.02	0.385

Data are presented as mean ± SD of relative IL-6 expression ($2^{-\Delta\Delta Ct}$). GAPDH was used as the reference gene. Different superscript letters indicate significant differences among groups at the same observation day ($p<0.05$).

Figure 2 illustrates the effect of rambutan honey–based toothpaste (TPRH) on IL-6 gene expression in gingival tissue of gingivitis-induced rats at different observation time points

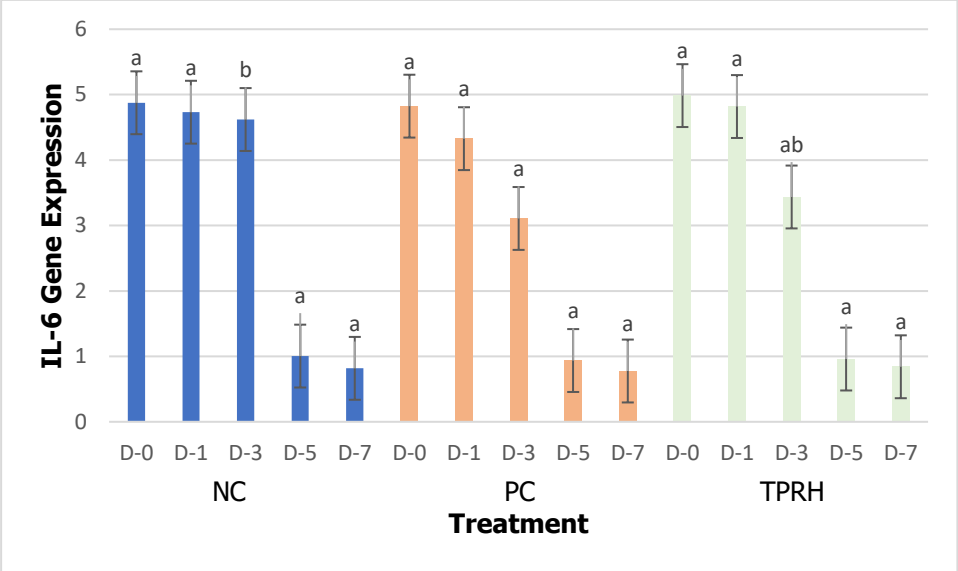

Figure 2. Effect of TPRH on IL-6 gene expression in gingival tissue of gingivitis rats.

Negative control (NC), positive control (PC), and rambutan honey toothpaste (TPRH) groups were analyzed. IL-6 gene expression was determined using relative quantitative real-time PCR and is presented as relative expression values ($2^{-}\Delta\Delta Ct$), expressed as mean ± standard deviation from three replicates. Different superscript letters indicate statistically significant differences among groups based on Tukey's post hoc test ($p < 0.05$).

On day 0 (baseline, prior to treatment), the relative IL-6 expression values in the NC, PC, and TPRH groups were 4.8761, 4.8242, and 4.9852, respectively. Statistical analysis showed no significant differences among groups ($p = 0.886$), indicating comparable baseline inflammatory status.

On day 1, IL-6 expression slightly decreased in all groups, with mean values of 4.7317 (NC), 4.3272 (PC), and 4.8183 (TPRH). However, no significant differences were observed among groups ($p=0.347$), although the PC group showed a numerically lower expression.

On day 3, a more pronounced reduction in IL-6 expression was observed, with mean values of 4.1698 (NC), 3.1078 (PC), and 3.4360 (TPRH). Statistical analysis revealed a significant difference among groups ($p = 0.038$), indicating differences in IL-6 gene expression during the acute inflammatory phase, with both PC and TPRH showing lower expression compared to NC.

By day 5, IL-6 expression had decreased markedly in all groups, reaching similarly low levels: 1.0043 (NC), 0.9364 (PC), and 0.9592 (TPRH). No statistically significant differences were observed among groups ($p = 0.794$), indicating that IL-6 suppression was no longer distinguishable at this later stage of inflammation.

Similarly, on day 7, IL-6 expression remained low across all groups, with mean values of 0.8171 (NC), 0.7762 (PC), and 0.8411 (TPRH). The absence of significant differences ($p = 0.385$) further suggests that group-related effects on IL-6 expression were limited to earlier observation periods.

Overall, IL-6 expression showed a decreasing trend from day 0 to day 7 in all groups. Significant differences among groups were observed only on day 3, while the lack of significant differences on days 5 and 7 indicate that IL-6 suppression occurs predominantly during the early inflammatory phase.

DISCUSSION

The present study demonstrated a progressive reduction in IL-6 mRNA expression in gingival tissues of gingivitis-induced rats from day 0 to day 7 across all experimental groups. No statistically significant differences were observed at baseline (day 0) and day 1 ($p>0.05$), indicating comparable inflammatory conditions prior to treatment. A significant intergroup difference was detected on day 3 ($p=0.038$), where both the commercial herbal toothpaste (PC) and rambutan honey toothpaste (TPRH) groups exhibited lower IL-6 expression compared to the negative control (NC). By day 5 and day 7, IL-6 levels converged to similarly low values in all groups, suggesting spontaneous resolution of gingival inflammation. This temporal pattern indicates that the primary effect of toothpaste interventions occurred during the early inflammatory phase, while later reductions likely reflect the natural healing process of gingival tissue.[18,19]

From a biological perspective, the relevance of the day 3 finding lies in the timing and magnitude of IL-6 suppression rather than sustained long-term differences. At this time point, both PC and TPRH demonstrated a greater reduction in IL-6 expression relative to NC, with the PC group showing the lowest mean value. However, the difference between PC and TPRH was modest and transient, and no significant differences persisted at later observation days. These findings suggest that rambutan honey toothpaste may contribute to early inflammatory modulation but does not confer a clear advantage over commercial herbal toothpaste in accelerating overall gingival healing.

Therefore, claims regarding accelerated healing should be limited to early-phase anti-inflammatory effects. Interleukin-6 is a pivotal pro-inflammatory cytokine produced by immune cells (macrophages, T and B lymphocytes) and resident gingival cells, including fibroblasts and epithelial cells, in response to microbial challenge and tissue injury.[20–22] Elevated IL-6 promotes inflammatory cell recruitment, increases vascular permeability, and activates downstream signaling pathways such as JAK/STAT and NF-κB, which are involved in connective tissue degradation and periodontal breakdown. [23,24] In periodontal disease, IL-6 is also linked to Th17 differentiation and RANKL-mediated osteoclastogenesis, contributing to alveolar bone resorption.[24,25] Accordingly, the high IL-6 expression observed at day 0 in this study reflects an active inflammatory phase and supports the validity of the ligature- and bacteria-induced gingivitis model.

The reduction in IL-6 expression observed at day 3 in the intervention groups is consistent with the early transition from acute inflammation toward resolution. Previous studies have demonstrated that decreases in IL-6 levels following periodontal therapy are associated with improvements in clinical parameters such as gingival bleeding and probing depth.[19,26] Although clinical indices were not evaluated in the present study, the early suppression of IL-6 mRNA suggests modulation of the inflammatory response during the critical initial phase of gingival healing.[8]

The observed IL-6 suppression in the TPRH group may be attributed to the bioactive constituents of rambutan honey, including flavonoids, phenolic acids, vitamin C, and antioxidant enzymes. These compounds have been reported to reduce oxidative stress, scavenge free radicals, and inhibit NF-κB activation, thereby downregulating pro-inflammatory cytokine transcription, including IL-6.[27–29] Experimental studies have also suggested that rambutan honey may enhance the expression of growth factors such as TGF-β, which supports fibroblast proliferation and extracellular matrix remodeling.[27,30] However, as the present study assessed only mRNA expression, these mechanisms remain hypothetical and require confirmation at the protein and histological levels.

By day 5 and day 7, IL-6 expression had decreased to similarly low levels across all groups, including the negative control, indicating that gingival inflammation in this model undergoes physiological resolution over time. The absence of significant intergroup differences at these later time points suggests

that IL-6 suppression becomes less discriminatory once the acute inflammatory phase subsides. This finding also indicates that IL-6 mRNA expression alone is a limited marker for complete inflammatory resolution or tissue repair.

Taken together, these findings emphasize that rambutan honey toothpaste exhibits anti-inflammatory potential by suppressing IL-6 expression, with a pattern comparable to commercial herbal toothpaste. Thus, toothpaste formulations containing rambutan honey may serve as a supportive or preventive therapeutic approach in managing gingivitis, mediated through suppression of NF-κB signaling, reduction of oxidative stress, and stimulation of TGF-β1–driven fibroplasia to promote gingival tissue repair.

Several limitations should be acknowledged. First, IL-6 was evaluated only at the gene expression level; protein-level confirmation using ELISA or immunohistochemistry was not performed. Second, no histological assessment (e.g., inflammatory infiltrate, epithelial integrity, fibroblast proliferation) or clinical parameters (e.g. gingival erythema or bleeding scores) were included, limiting conclusions regarding actual periodontal healing. Finally, the modest and transient difference between PC and TPRH underscores the need for cautious interpretation of clinical relevance. Future studies incorporating additional inflammatory markers, protein-level validation, histological scoring, and clinical indices are required to strengthen the evidence for the anti-inflammatory potential of rambutan honey–based toothpaste.

CONCLUSION

Rambutan honey toothpaste reduced IL-6 expression in gingival tissues of gingivitis-induced rats. A significant decrease was observed on day 3 in both the commercial herbal toothpaste and rambutan honey toothpaste groups compared to the negative control, indicating suppression of inflammation during the acute phase. The implication of this study is that rambutan honey toothpaste may be considered a potential natural adjunctive therapy for the management of gingivitis, particularly as an alternative oral hygiene product derived from natural ingredients. However, further studies incorporating clinical, histological, and protein-level assessments are required.

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Author Contributions: research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, ERY and RYP.; methodology ERY, RYP, RPP., and WW; software RYP.IYN; validation, ERY.,WW., and RPP.; formal analysis,RYP; investigation, ERY, RYP, RPP., and WW; resources, RYP.; data curation, RYP.; writing original draft preparation,RYP.; writing review and editing, ERY.; visualization, RYP.; supervision,ERY.; project administration RYP; funding acquisition, RYP, ERY. All authors have read and agreed to the published version of the manuscript.", please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.
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