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Original Research Article

Epigallocatechin-3-gallate Modulates NF-κB in *Porphyromonas gingivalis*-Induced Periodontitis

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ABSTRACT

Periodontitis is a severe bacterial infection that affects soft tissues around teeth. *Periodontitis* is caused by a Gram-negative anaerobic bacterium called *Porphyromonas gingivalis* (*P. gingivalis*), which promotes the expression of nuclear factor kappa *β* (NF-κ*β*). In recent years, medical herbs such as green tea containing Epigallocatechin-3-Gallate (EGCG) have been employed extensively in anti-inflammatory and antibacterial studies due to their low toxicity and strong efficacy based on their multitarget therapy. The mucoadhesive drug delivery system adheres to the surface of the oral mucosa, providing sustained release, enhancing local effects, bypassing first-pass metabolism, and minimizing systemic side effects. This study aimed to investigate the impact of an EGCG-loaded mucosal gingival patch on periodontitis caused by *P. gingivalis*. Periodontitis was induced in Wistar rats by administering 0.03 mL containing 10^{10} (CFU) in phosphate-buffered saline (PBS) into the incisive gingival sulcus of the mandible of the anterior teeth. The rats with induced periodontitis received the treatment as mucoadhesive gingival patches loaded with EGCG (GP-EGCG), doxycycline (GP-doxy), and blank patches for one hour daily over 3, 5, 7, 14, and 21 days. Immunohistochemical analysis was performed on the anterior inferior gingival sulcus after the treatment. Results showed that the treatment of periodontitis with GP-EGCG significantly lowered NF-κB expression compared to the control groups following treatment during 3, 5, 7, 14, and 21 days ($p < 0.05$). EGCG has effectively reduced inflammatory markers in *P. gingivalis*-induced periodontitis. These findings suggest that the treatment based on EGCG holds promising potential for managing periodontitis, providing an alternative approach to traditional therapies.

Keywords: Epigallocatechin Gallate, Medicine, Mucoadhesive Gingival Patches, Nuclear factor kappa *β*, Periodontitis, *P. gingivalis.*

Introduction

Medicinal plants and alternative therapies offer a rich source of bioactive compounds that are increasingly utilized in pharmaceuticals to treat infections and inflammatory conditions. Green tea, derived from the *Camellia sinensis* plant, has gained considerable attention for its proven health benefits, particularly its potent antibacterial properties. With its wide therapeutic applications, variability in bioactive compounds, and effectiveness in addressing oral diseases, green tea extract shows promise as a preferred alternative treatment option. [1]An estimated 2.5 million tons of green tea leaves are harvested annually, comprising approximately 20 % of the total tea production.

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Due to its wide-ranging health advantages, green tea has been a traditional beverage for centuries in India, China, Japan, and Thailand. [2] There is an agreement that the positive effects of green tea are primarily due to its polyphenols. It contains four primary polyphenolic compounds called catechins, including 1. (-)-epicatechin (EC), 2. (-)-epicatechin-3-gallate (ECG), 3. (-)-epigallocatechin (EGC), and 4. (-)-epigallocatechin-3-gallate (EGCG). [3] EGCG is the most prevalent polyphenol in green tea. It exhibits various therapeutic effects, including anti-inflammatory, antioxidant, antibacterial, and anticarcinogenic properties. EGCG's ability to form complexes and biocompatibility make it a promising candidate for enhancing local drug delivery systems. Recent scientific studies have made significant progress in discovering and developing innovative drug-delivery methods based on EGCG. [4,5]

Oral drug administration often relies on the use of mucosal drug delivery systems. These systems are designed to adhere to the oral mucosal surfaces, allowing sustained drug release and enhanced local effects. Mucoadhesive formulations prolong drug contact time, improve bioavailability, minimize systemic side effects, bypass the first-pass metabolism and improve drug efficacy. As a result, mucoadhesive drug delivery systems represent a promising approach for optimizing the therapeutic potential of orally administered drugs.

The mucoadhesive gingival patch has many notable applications in dentistry. Specifically, these patches are designed to release drugs locally and effectively target fungal infections such as oral candidiasis. Besides, they have been innovatively utilized in treating periodontitis, representing a significant advance in dental care. [6,7]

Periodontitis is a chronic inflammatory immune-related disease caused by bacterial plaques on teeth of multifactorial aetiology. The primary bacteria in the plaque, such as *Porphyromonas gingivalis (P. gingivalis)*, are harmful gram-negative and responsible for initiating destructive processes. These bacteria produce lipopolysaccharides that trigger host responses. [7,8] These reactions involve stimulating fibroblasts and endothelial cells, attracting inflammatory cells like macrophages and lymphocytes, and releasing inflammatory mediators and cytokines, leading to damage in periodontal tissues. *P. gingivalis* targets the upregulation of nuclear factor kappa β (NF-κB) expression and promotes the production of proinflammatory cytokines. [9] NF-κB is a vital transcription factor in inflammation, tissue damage, and bone degradation. Periodontal patients suffer from inflammation of soft tissues, collagen breakdown, and bone resorption. In individuals with chronic periodontitis, the activation of NF-κB is significantly higher than in those without periodontitis. Additionally, NF-κB has been shown to regulate the osteogenesis of periodontal ligament stem cells when subjected to inflammation. Hence, NF-κB could potentially impact the development of periodontitis through various pathways. [10]

Periodontal therapy primarily relies on mechanical debridement techniques such as scaling and root planning, often complemented by surgical interventions to address subgingival microbial biofilms. However, integrating antimicrobial agents, particularly for eradicating pathogens like *P. gingivalis*, has emerged as a crucial adjunct to standard treatment protocols. Hence, Mucogingival patches offer a promising therapeutic modality due to their safety profile and potential to enhance treatment outcomes. [11] This study serves as a continuation of previous studies aimed to confirm the effectiveness of treating periodontitis *in vivo*. [6,7,12] This study aims to investigate the impacts of an EGCG-loaded mucosal gingival patch on periodontitis Wistar rat's models induced by *P. gingivalis* by expression NF-κB an inflammatory marker.

Materials and Methods

Preparation of EGCG and Mucoadhesive Gingival Patches

The EGCG extracted from *Camellia sinensis* (Xi'an Rongsheng Biotechnology Co., Ltd., Shaanxi, China, with a concentration of <98%) was formulated by combining it with 80% polyethylene glycol (PEG) 400 sourced from Schuchardt OHG, Germany, and 20% PEG 4000 obtained from Sigma-Aldrich, St. Louis, USA.

Gingival patches with mucoadhesive properties were prepared for the treatment and control groups using solvent casting. These patches included formulations containing EGCG, doxycycline, and blank patches. In the blank patch groups, carboxymethyl cellulose sodium (CMC-Na) [0.6 g] was dissolved in warm distilled water, followed by the addition of propylene glycol (PG) [1 g] with constant stirring. The resulting mixture was poured into Petri dishes, deaerated, and oven-dried to yield patches with a uniform thickness of 0.3 mm. Similarly, patches containing EGCG [15 mg] or doxycycline [100 mg] followed the same procedure, adding the respective drug and 1 g of PEG for uniformity. [12]

Animal models

This protocol was approved by the Airlangga University Ethics Committee, Surabaya, Indonesia, Ref no: (466/HRECC.FODM/X/2020), and complied with the National Animal Care Guidelines. The experiment involved healthy male Wistar rats (Rattus norvegicus), aged (5-6 months) and weighing (250-300 g), from the Animal Laboratory of the Faculty of Veterinary Medicine, Airlangga University. The total number was 45 samples, with 15 groups, each consisting of 3 replications. Samples were grouped based on type and duration of treatment. After a one-week acclimatization period, the rats were categorized into three groups, as mentioned earlier. Periodontitis was induced by injecting 0.03 ml containing 10^{10} CFU of *P. gingivalis* into the mandibular right and left incisive gingival sulci of the animals' anterior teeth every two days for 14 days [13]. This procedure was performed using a disposable 0.5 cc syringe. After the development of periodontitis, each group of animals was treated under anaesthesia induced by a ketamine (10%) intramuscular injection at a dosage of 0.1 ml per 100 g body weight. Gingival patches (GP) of EGCG, doxycycline antibiotic, and blank patches were placed in the periodontitis area for an hour daily. Animals were euthanized with ketamine (0.4 mL/100 g) 3, 5, 7, 14, and 21 days after treatment, after which a biopsy of the mandibular anterior region was performed. Immunohistochemistry (IHC) was subsequently conducted on the anterior mandible of rats to investigate the expression of NF-κB using a Nikon light microscope (model H600L) from Tokyo, Japan, at a magnification of 400x, examining five different fields of view. [3]

Statistical Analysis

Statistical analyses were conducted using SPSS version 25. Variations in NF-κB expression were assessed using an independent Mann-Whitney test. A p-value below 0.05 was considered statistically significant for each group.

Results and Discussion

EGCG, a potent bioactive compound, exhibits remarkable effects on inflammatory and proinflammatory processes. [3] This study demonstrates that GP-doxy and GP-EGCG treatments effectively reduce NF-κB expression in periodontal tissues of Wistar rats, with GP-doxy showing the highest reduction by the twenty-first day Figure 1. The mucoadhesive gingival patches used in this study allow for the direct delivery of doxycycline and EGCG, leveraging their anti-inflammatory properties to target periodontal inflammation efficiently Figure 2. After three days of treatment, there was a significant reduction in NF-κB expression, with the GP-doxy group exhibiting the most significant decrease, followed by the GP-EGCG group. As shown in Table 1, the average expression of NF-κB continued to decrease at subsequent observation points on days 3, 5, 7, 14, and 21, highlighting the sustained anti-inflammatory impact of EGCG. This finding aligns with previous studies that have demonstrated the anti-inflammatory properties of doxycycline and EGCG. [3,14]

Table 1: Average amount of NF-κB expression in each sample group

	Day 3	Day 5	Day 7	Day 14	Day 21
Control blank	9.33 ± 2.082	10.33 ± 2.082	12.00 ± 2.000	11.67 ± 1.528	11.67 ± 1.155
GP-doxy	5.33 ± 1.155	5.00 ± 1.732	4.00 ± 1.000	3.33 ± 1.528	2.33 ± 0.577
GP-EGCG	8.00 ± 1.000	7.00 ± 1.000	5.00 ± 1.000	5.00 ± 1.000	4.00 ± 1.000

Doxycycline, a well-known antibiotic, has been shown to inhibit matrix metalloproteinases and reduce the expression of proinflammatory cytokines, contributing to its effectiveness in reducing periodontal inflammation. [15] Similarly, EGCG, a major polyphenol in green tea, has been documented to possess strong anti-inflammatory and antioxidant properties. A study by Fan et., (2023) demonstrated that EGCG significantly reduced NF-κB activation and the production of inflammatory mediators in a model of periodontal disease, supporting the results observed in this study. [16]

Furthermore, the use of mucoadhesive gingival patches for the delivery of doxycycline and EGCG ensures localized and sustained release of these agents, thereby enhancing their therapeutic effects. This method of delivery has been shown to maintain higher concentrations of the drug at the site of inflammation, resulting in more effective suppression of inflammatory pathways. [17]

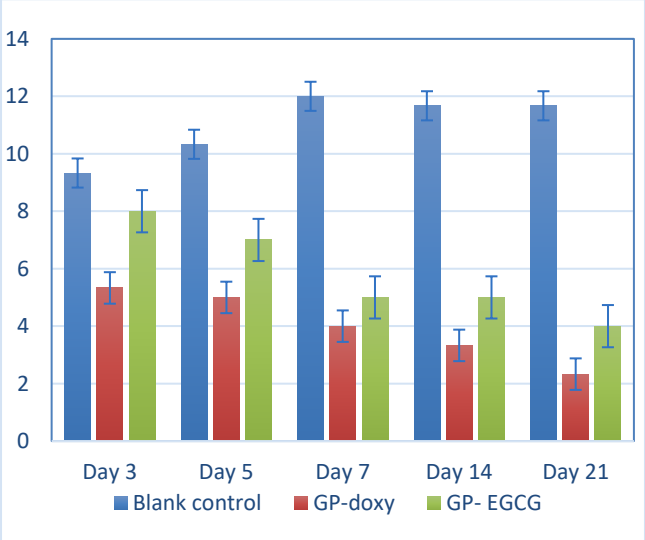

Figure 1: Mean X ± SD of NF-κB expression

The study revealed in Table 2 that both GP-doxy and GP-EGCG treatments significantly reduced NF-κB expression compared to the GP-blank group, particularly on the seventh, fourteenth, and twenty-first days. Although there was a notable reduction on the third day with GP-doxy showing a greater decrease than GP-EGCG ($p = 0.04$), no significant differences were observed between the two active treatment groups on the fifth, seventh, fourteenth, and twenty-first days. These findings indicate the sustained efficacy of both treatments in reducing inflammation, with no significant difference between the anti-inflammatory effects of GP-doxy and GP-EGCG. The significant differences observed compared to the GP-blank group underscore the therapeutic potential of these mucoadhesive gingival patches.

The decrease in NF-κB expression observed with EGCG administration is consistent with other studies. Machova Urdzikova et al., (2017) found that EGCG can reduce NF-κB expression within 72 hours. [5] Furthermore, Saito et al., (2014) reported a reduction in NF-κB expression until day 14, with no significant difference between days 7 and 14. [18] However, Machova Urdzikova et al., (2017) demonstrated that the expression of pro-inflammatory cytokines could continue to decrease for up to 28 days after EGCG administration [5].

NF-κB is a transcription factor responsible for regulating genes associated with chronic inflammation-related diseases. While inactive in the cytoplasm of unstimulated cells, NF-κB relocates to the nucleus upon activation by external stimuli, leading to the expression of pro-inflammatory genes. Increased NF-κB expression is observed in individuals with chronic periodontitis, suggesting it is a potential therapeutic target for treating periodontitis [19]. Previous study by Saito et al., (2014) demonstrated that EGCG reduces inflammatory markers such as COX-2, NF-κB, and TNF-α over 14 days. This study utilized microscopic evaluation of NF-κB expression in macrophage cells from periodontal tissue biopsies of Wistar rats, stained using the IHC method.

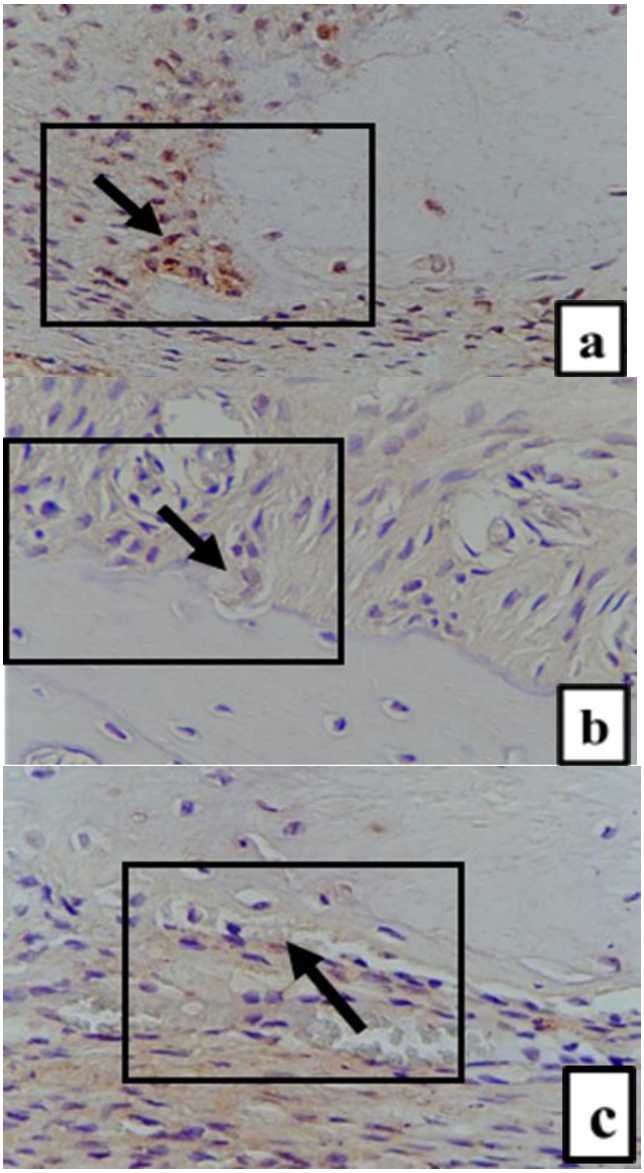

Figure 2: Immunohistochemistry images (400x magnification). Black arrows indicate macrophage cells expressing NF-κB (a) Blank group, (b) GP-doxy, and (c) GP-EGCG.

The highest average number of NF-κB expressions was observed in the blank group on the seventh day, likely due to the ongoing inflammatory process and the absence of active ingredients in the patch to mitigate inflammation. Antigens activate immune cells during the initial immune response phases (days 0 to 7), increasing pro-inflammatory factors like NF-κB. [20]

GP-doxy showed the lowest average NF-κB expression by the twenty-first day, indicating its effectiveness in reducing periodontitis-related inflammation. Doxycycline inhibits LPS-induced phosphorylation of p38 MAP kinase and the translocation of NF-κB, leading to a significant decrease in NF-κB expression than the blank group. [21] Over time, the decrease in NF-κB and other pro-inflammatory cytokines, such as IL-1β and TNF-α, suggested that the GP-doxy patch targets the inflammatory response and aligns with the natural healing process. [3,12] EGCG, derived from green tea leaf extract, is known for its antioxidant, anti-inflammatory, and antimicrobial effects.3,22

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Table 2: Mann-Whitney test results for NF-κB expressi

Significant	3 GP	3 GP-doxy	3 GP-EGCG	5 GP	5 GP-doxy	5 GP-EGCG	7 GP	7 GP-doxy	7 GP-EGCG	14 GP	14 GP-doxy	14 GP-EGCG	21 GP	21 GP-doxy	21 GP-EGCG
3 GP															
3 GP-doxy	0.046*														
3 GP-EGCG	0.376	0.046*													
5 GP	0.376	0.046*	0.184												
5 GP-doxy	0.072	0.814	0.072	0.046*											
GP-EGCG	0.184	0.105	0.261	0.077	0.178										
7 GP	0.184	0.046*	0.050*	0.376	0.046*	0.050*									
7 GP-doxy	0.050*	0.178	0.050*	0.050*	0.487*	0.050*	0.050*								
7 GP-EGCG	0.050*	0.637	0.050*	0.050*	0.817	0.077	0.050*	0.261							
14 GP	0.184	0.046	0.050*	0.376	0.046	0.050*	0.822	0.050*	0.050*						
14 GP-doxy	0.050*	0.121	0.050*	0.050*	0.268	0.050*	0.050*	0.500*	0.184	0.050*					
14 GP-EGCG	0.050*	0.637	0.050 *	0.050*	0.817	0.077	0.050*	0.261	1.000	0.050*	0.184				
21 GP	0.105	0.043	0.046	0.487	0.043	0.046	0.825	0.046	0.046*	1.000	0.046*	0.046*			
21 GP-doxy	0.046*	0.043*	0.046*	0.046*	0.043*	0.046*	0.046*	0.072	0.046*	0.046*	0.346	0.046*	0.043*		
21 GP-EGCG	0.050*	0.178	0.050*	0.050*	0.487	0.050*	0.050*	0.000*	0.261	0.050*	0.500*	0.261	0.046	0.072	

* A significant difference (A p-valu < 0.05)

When administered via a mucoadhesive gingival patch, EGCG is directly absorbed into the circulatory system, providing localized effects on periodontal tissues. This delivery method aligns with previous findings, which demonstrated that mucoadhesive patches enhance the release of active drug ingredients, offering a viable alternative to systemic drug delivery methods. [23] This study used a single concentration of EGCG, which was chosen based on previous studies. [3] EGCG binds to 67LR receptors on immune cells like macrophages, inhibiting NF-κB activation [19]. EGCG was more effective in reducing NF-κB expression than mucoadhesive gingival patches, and this difference was significant. On the other hand, doxycycline also reduced the amount of NF-κB expression compared to EGCG, but the difference was insignificant.

Periodontitis results from dysregulated immune responses to external stimuli. Current therapies include non-surgical periodontal treatments like scaling and root planning, local drug delivery, and systemic chemotherapeutic agents as adjuncts. [6,7] Imbalances in cytokine networks are linked to inflammatory disorders. Recent data suggest that plant-derived compounds exhibit anti-inflammatory properties, primarily targeting cytokines, chemokines, or adhesion molecules. [24] Therefore, complementary and alternative medicine, including plant-derived compounds, could be valuable for treating inflammatory conditions such as periodontitis. [25] Moreover, due to its antimicrobial and anti-inflammatory properties, EGCG can reduce plaque and calculus formation associated with several oral conditions, including gingivitis, dental caries, and halitosis. Some studies suggest that using an EGCG-based mouth rinsing can be advantageous for treating periodontal issues and managing oral malodor. [25–28]

A significant limitation of this study is the small sample size and dosage used, which indicates the possibility of expanding future investigations. More studies should be conducted to examine the effects of EGCG at different concentrations to determine the best results and strengthen the reliability of the findings. Furthermore, studying additional variables or other effects of EGCG, besides its anti-inflammatory properties, would contribute to a more comprehensive understanding of its therapeutic potential.

Conclusion

GP-EGCG mucoadhesive gingival patches present an effective therapeutic intervention for periodontitis by decreasing NF-κB expression. Future research should focus on refining dosage, exploring broader therapeutic effects, and confirming these findings in clinical settings to fully realize these innovative treatments' potential.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors declare that the content presented in this article is entirely original, and they accept responsibility for any claims regarding its content.

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