Archives of Oral Biology 137 (2022) 105396

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Nigella sativa toothpaste promotes anti-inflammatory and anti-destructive effects in a rat model of periodontitis

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ARTICLE INFO

Keywords:
Black cumin
Antibacterial toothpaste
Dental plaque
Porphyromonas gingivalis
Infectious disease

ABSTRACT

Objective: Periodontitis is an infectious disease that results in gingiva tissue damage. This study aimed to evaluate the effects of *Nigella sativa* (*N. sativa*) toothpaste in a periodontitis tissue repair based on inflammation and periodontal extracellular matrix in vivo.

Design: The periodontitis disease model was developed using Wistar rats infected with *Porphyromonas gingivalis* (*P. gingivalis*). The rats were divided into three main groups as follows: those that did not receive any toothpaste treatment; those that were treated with *N. sativa* toothpaste twice a day (simultaneously with *P. gingivalis* induction); and normal healthy rats. The rats were sacrificed after 1 and 7 days of animal modeling. The number of inflammatory cells, matrix metalloproteinase (MMP)1 + and MMP8 + cells, levels of cytokines (interleukin-1β (IL-1β) and prostaglandin E2 (PGE2)) and density of collagen type 1 were determined in the gingival tissues of the rats.

Results: The rats treated with *N. sativa* toothpaste had significantly lower numbers of neutrophils, macrophages and lymphocytes than the non-treated rats after 1 and 7 days of treatment; likewise, the levels of IL-1β and PGE2 were lower in the treated experimental rats. In addition, the group treated with *N. sativa* toothpaste had fewer numbers of MMP1 + and MMP8 + cells and higher collagen density after 1 and 7 days of administration.

Conclusions: N. sativa toothpaste exhibited anti-inflammatory effects by reducing both inflammatory cell count and activity. Additionally, *N. sativa* toothpaste demonstrated anti-destructive effects on the periodontal extracellular matrix. Thus, *N. sativa* toothpaste might be potentially used for the management of periodontitis.

1. Introduction

Periodontitis is a multifactorial chronic illness induced by oral bacteria, characterized by a host-mediated inflammation in the periodontal tissues accompanied by dysbiotic plaque biofilms, which leads to the gradual degradation of the tooth-supporting apparatus and loss of periodontal attachment. It is one of the most common causes of tooth loss and can affect the masticatory efficiency, appearance, self-esteem and quality of life of the individual (Muñoz-Carrillo et al., 2019). Periodontal disease affects 20–50% of the population worldwide (Sanz, D'aiuto, Deanfield, & Fernandez-Avilés, 2010). The average proportion increase in the global burden of periodontitis, oral cancer and dental caries from 1990 to 2010 was reported to be 45.6% (Jin et al., 2016). In developing countries such as Indonesia, the prevalence of periodontitis was expected to be higher than 70% (Susanto et al., 2011).

A variety of salivary and serum pro-inflammatory biomarkers were found in periodontitis patients. Isola, Polizzi, Santonocito, Alibrandi, and Williams (2021) found that an elevated serum and salivary Nod-like receptor family pyrin domain-containing protein-3 (NLRP3), a caspase recruitment domain (ASC), caspase-1, and interleukin (IL)− 1β were

Abbreviations: COL1, collagen type 1; IgA, immunoglobulin A; IHC, immunohistochemistry; IL-1β, interleukin-1β; MMP1, matrix metalloproteinases 1; MMP8, matrix metalloproteinases 8; *N. sativa*, *Nigella sativa*; *P. gingivalis*, *Porphyromonas gingivalis*; PGE2, prostaglandin E2; TNF-α, tumor necrosis factor-α.

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https://doi.org/10.1016/j.archoralbio.2022.105396
Received 7 July 2021; Received in revised form 3 February 2022; Accepted 6 March 2022
Available online 9 March 2022

found in periodontitis patients. This was also similar in the IL-6 (Isola, Lo Giudice et al., 2021) and other pro-inflammatory cytokines levels (Liao, Fei, Shen, Yin, & Lu, 2014; Rangbulla, Nirola, Gupta, & Batra, 2017). It has been reported that recruitment in NLRP3-caspase 1 leads to the recruitment of inflammatory cells such as neutrophils (Strowig, Henao-Mejia, Elinav, & Flavell, 2012). The inflammatory cells then will release pro-inflammatory cytokines and mediators, such as tumor necrosis factor-α (TNF-α), IL1β, and prostaglandin E2 (PGE2). Moreover, macrophages and lymphocytes contribute to the production of collagenases, including matrix metalloproteinases 1 (MMP1) and 8 (MMP8), which degrade the organic matrix in periodontal tissues (Kinney et al., 2014; Rangbulla et al., 2017). Other proteins such as Galectin− 3 dan asymmetric dimethylarginine (ADMA) also involved in periodontitis progression and its association with cardiovascular diseases (Isola, Polizzi, Alibrandi, Williams, & Lo Giudice, 2021; Isola et al., 2020). Considering their importance in periodontitis pathophysiology, inflammation factors are required as biomarkers for periodontitis.

The management of periodontitis varies from a lifestyle change, local and systemic pharmacotherapy, and surgery. A meta-analysis reported that scaling, root planing, and systemic antibiotic therapy led to beneficial clinical effects in aggressive periodontitis (Gonc, 2015). Even so, there are various side effects of antibiotics therapy in periodontitis, mostly related to microbiologically adverse effects and increase in bacterial resistance (Jepsen & Jepsen, 2016). Besides, regenerative surgical treatment such as bone graft is essential to replace the bone destroyed (Khotib et al., 2021). Despite its success in dental implantation, biomaterials used as bone grafts are also reported to cause plaque accumulation, gingival inflammation, and the potentially toxic effects caused by released components (Isola, 2021).

Periodontitis is caused by microbial biofilms formed by oral bacteria such as *Porphyromonas gingivalis* (*P. gingivalis*) that occur due to improper oral health (Mekhemar, Hassan, & Dörfer, 2020). Maintenance of oral hygiene by methods such as tooth brushing is an inexpensive but effective way to prevent and treat periodontitis (AlAttas, Zahran, & Turkistany, 2016). Nahak, Tedjasulaksana, and Raiyanti (2018) reported that tooth brushing with antibacterial toothpaste reduced the number of periodontal pathogens in the dental plaque. *Nigella sativa* (*N. sativa*) is a medicinal plant with a variety of pharmacological effects (Ahmad et al., 2013; Nurhan, Gani, Budiatin, Siswodihardjo, & Khotib, 2021). A study by Senthilnathan, Ilango, Abiram, Vummidi, and Mahalingam (2020) showed that *N. sativa* had an antibacterial effect against periodontitis-causing bacteria, *P. gingivalis* and *Prevotella intermedia*. In addition, we have recently shown the non-toxic effects of toothpaste containing *N. sativa* and 2% sodium lauryl sulfate and non-sodium lauryl sulfate on fibroblasts (Setiawatie et al., 2021). These findings indicate that *N. sativa* can be used as the main ingredient in toothpastes for adjuvant therapy in periodontitis.

Considering the incidence of periodontitis, there should be an effective and inexpensive way to prevent and treat this disease. The antibacterial effect of *N. sativa* has been widely reported (Bakathir & Abbas, 2011; Salem et al., 2010; Senthilnathan et al., 2020). However, to the best of our knowledge, there is no information regarding the anti-inflammatory and anti-destructive effects of toothpaste containing *N. sativa*. Thus, this study aimed to evaluate the effects of toothpaste containing *N. sativa* in the repair periodontitis tissue based on the neutrophil, macrophage, and lymphocyte cell counts, collagen type 1 fibers (COL1) density, (IL)− 1β and PGE2 levels, as well as MMP1 + and MMP8 + cells in vivo. The results of this study will contributes in finding an effective and inexpensive ways in periodontitis management.

2. Materials and methods

2.1. Toothpaste fabrication

Na CMC (also known as sodium carboxymethyl cellulose salt) was placed in a beaker containing hot distilled water (80 °C) and stirred. Next, cold distilled water was added to the Na CMC mixture and stirred until homogenous. Titanium dioxide was crushed in a mortar, mixed with $CaCO_3$ until homogeneous and placed in a bottle glass containing saccharine. This mixture was then combined with *N. sativa* extract and stirred until homogeneous. The Na CMC mucilage was added to the mixture and homogenized. Finally, sorbitol, aqua, silica, sodium monofluorophosphate, sodium benzoate, flavor, allantoin, methylparaben and CL 42090 (colorant) were added to the mixture and stirred to form a paste.

2.2. In vivo study

Thirty-six male Wistar rats (*Rattus norvegicus*) aged 5–6 months (bodyweight, 250–350 g) were adapted to the laboratory environment for at least 1 week and housed under standard laboratory conditions. All procedures conducted on animals were approved by the Health Research Ethical Clearance Commission at the Faculty of Dental Medicine, Universitas Airlangga (approval number: 564/HRECC.FODM/XII/2020). The periodontitis model was developed as described by Budhy, Arundina, Surboyo, and Halimah (2021). Briefly, *P. gingivalis* (Pg ATCC 33277 PK/5, Thermo Scientific; 1 × 10 colony-forming units in 20 μL of PBS) was injected into the gingival sulcus between the two lower mandibular incisors using a 0.5-mL syringe. The injection was performed every 3 days for 14 days. The initial signs of periodontitis, which included a reddish pigmentation of the gingiva, swelling of the interdental incisive central mandibular area and interdental resorption (during histopathological investigation) were observed from the 14th day.

One milligram of *N. sativa* toothpaste was administered twice a day simultaneously with the induction of *P. gingivalis* in the gingival sulcus of the mandibular incisors. The animals were randomly divided into three main groups. The normal group were healthy controls, and the two remaining groups were used as periodontitis model groups. The periodontitis model groups were further subdivided into four groups as follows: PG day 1 group (the rats were sacrificed after day 1); PG + toothpaste day 1 group, which comprised rats that received *N. sativa* toothpaste and were sacrificed after day 1; PG day 7 group (the rats were sacrificed after day 7); and PG + toothpaste day 7 group, which comprised rats that received *N. sativa* toothpaste treatment and were sacrificed after day 7. All subgroups were contained five rates. The rats were euthanized using chloroform. The gingival tissues from the incisors to the molars were collected and fixed in 10% neutral buffered formalin for 3 days.

2.3. Histological staining

Histological staining was performed to determine the number of inflammatory cells and the collagen density. The tissue samples were implanted in a paraffin block. The paraffin blocks were made by dehydrating the tissue in various grades of ethanol (70–100%, 60 min each). The tissues were then washed with xylol (3 times, 15 min each), followed by liquid paraffin infiltration and transfer (3 times, 60 min each in a 60 °C incubator). A rotary microtome was used to cut the paraffin blocks (thickness, 4–6 μm). The slides were stained with haematoxylin–eosin (Budiatin et al., 2021) and Masson trichrome, according to the standard procedures.

2.4. Immunohistochemistry

The expression of MMP1 and MMP8 was investigated via immunohistochemistry (IHC). The paraffin blocks were proceed the same as in the histological staining. Briefly, after being implanted in paraffin blocks, the gingiva tissue was dehydrated and washed with xylol. Moreover, the samples were infiltrated, transferred, and cut into paraffin blocks. The IHC procedure was conducted as described by Budiatin et al. (2021). Briefly, the histological slide was deparaffinized

using xylol (3 times, 5 min), followed by rehydration using alcohol (absolute to 70%, 4 min each) and rinsed under running water (5 min). The slide was blocked using endogenous peroxide 0.5% (5 min) and rinsed with running water (5 min). Antigen retrieval was performed in a de-cloaking chamber; following which, the specimen was cooled for approximately 20 min, washed with PBS (3 min) and snipper-blocked for 15 min. The slide was then incubated with rabbit anti-rat MMP1 (BS-0463R, Bioss, Massachusetts, USA, 1:250 dilution) or rabbit anti-rat MMP8 (orb214259, Biorbyt, Cambridge, UK, 1:250 dilution) for 60 min and washed with PBS for 3 min. Moreover, the Trekkie Universal link

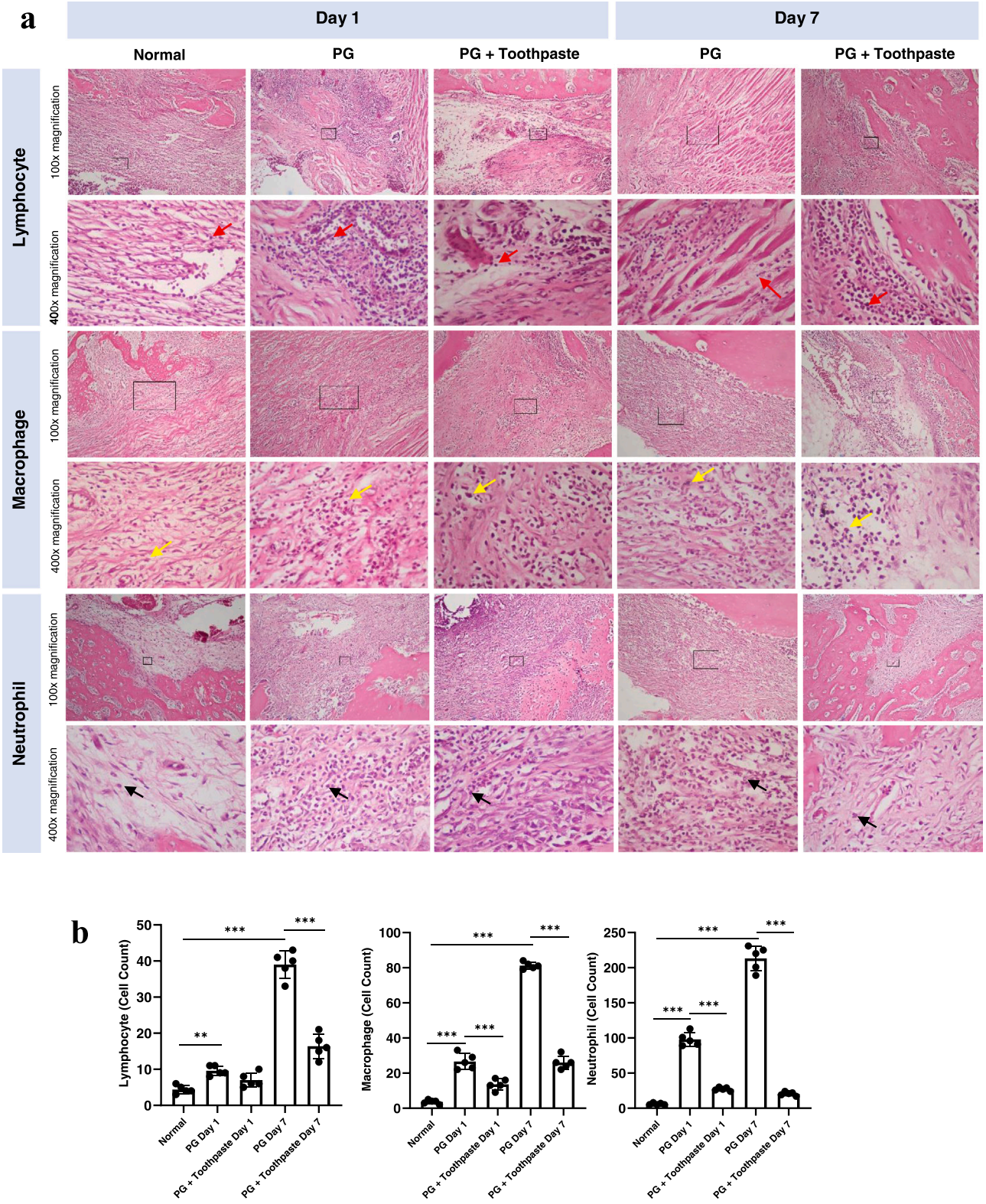

Fig. 1. Representative histological images of gingiva tissues stained with haematoxylin–eosin (total magnification, 100× and 400×), lymphocyte (red arrow), macrophage (yellow arrow), and neutrophil (black arrow) (a). Histophotometric quantification of neutrophils, macrophages and lymphocytes. Each bar represents the mean cell count ± standard deviation (SD) of five different visual fields in five rats. $^{**}p < 0.01$, $^{***}p < 0.001$ based on the one-way ANOVA test (b).

was carried out on the slide (20 min), and the slide was rewashed with PBS for 3 min. Trekavidin-HRP Label was applied to the slide (10 min); following which, the specimen was washed with PBS (3 min). The slide was reacted with chromogen DAB + buffer substrate (2–5 min) and washed with running water (5 min). After counterstaining with haematoxylin (1–2 min) and washing under running water (2 times, 5 min each), the slide was dehydrated with alcohol (70% to alcohol absolute, 5 min each), cleared in xylol (3 times, 5 min each), mounted (Ecomount) and covered with a coverslip.

2.5. Histophotometry

Quantitative assessment of inflammatory cells in the gingival connective tissue on the lingual side was performed by an investigator blinded to the animal groups as described by Sha, Garib, Azeez, and Gul (2020). A light microscope with an image analyzing system (Motic, ToupTek, ToupView) was used to perform the quantification (magnification, 400 ×). A grid of 16 squares was used to split each collected image. The mean ± standard deviation (SD) values of the neutrophils, macrophages and lymphocytes were determined. The density of the collagen fibers (%) was determined by calculating the area formed by the collagen fibers at the healing center and dividing it by the total area of measurement (Budi & Astuti, 2019). On the basis of the IHC results, the mean percentage ± SD values of immunopositive cells expressing MMP1 and MMP8 were determined.

2.6. Enzyme-linked immunosorbent assay

Blood (3 mL) was collected from each rat via intracardiac puncture before sacrifice, transferred to empty tubes and centrifuged for 10 min at 3000 rpm. The obtained serum sample was used to measure the levels of PGE2 and IL-1β. The procedures were conducted using the Prostaglandin E2 Assay Kit (PKGE004B, R&D Systems, Minneapolis, MN, USA) and Rat IL-1β/IL-1F2 Immunoassay Kit (RLB00, R&D Systems, Minneapolis, MN, USA), according to the manufacturers' instructions.

2.7. Statistical analysis

One-way analysis of variance was used to analyze the data; the significance level was set at 0.05. The statistical tests were performed using SPSS version 24.0 (IBM Corporation, Armonk, NY, USA).

3. Results

The numbers of neutrophils, macrophages and lymphocytes were qualitatively (Fig. 1a) and quantitatively ($p < 0.001$; Normal vs. PG Day 1, Normal vs. PG Day 7; Fig. 1b) increased after induction with *P. gingivalis*. Furthermore, induction with *P. gingivalis* decreased the COL1 density ($p < 0.001$, Normal vs. PG Day 1, Normal vs. PG Day 7; Fig. 2). The number of neutrophils was decreased after days 1 and 7 of treatment with *N. sativa* toothpaste ($p < 0.001$; Fig. 1b). Likewise, the administration of *N. sativa* toothpaste resulted in a decrease in macrophage cell counts after the first and seventh days of treatment ($p < 0.001$; Fig. 1b). In the case of the lymphocytes, a decrease in number was noted after the seventh day of treatment compared to the group without treatment ($p < 0.001$; Fig. 1b); this value was not significantly different from that in the normal group ($p = 0.125$; Normal vs. PG Day 1; Fig. 1b). Significant increases in the density of COL1 were observed after days 1 and 7 of using *N. sativa* toothpaste ($p < 0.001$; Fig. 2).

Furthermore, there was a significant increase in IL-1β serum concentration in the PG Day 1 and PG Day 7 groups compared with those in the normal group ($p < 0.001$; Fig. 3a). This indicated that the periodontitis model was successful based on the pro-inflammatory cytokine levels. However, treatment with the *N. sativa* toothpaste decreased the IL-1β serum concentration at both time points ($p < 0.01$, Normal vs. PG + Toothpaste Day 1, Normal vs. Toothpaste Day 7; Fig. 3a). Similarly, *P. gingivalis* induction increased PGE2 serum concentration in the PG Day 1 and PG Day 7 groups compared with those in the normal group ($p < 0.001$; Fig. 3b), whereas treatment with *N. sativa* toothpaste, decreased PGE2 serum concentration at both time points ($p < 0.01$, Normal vs. PG + Toothpaste Day 1, Normal vs. Toothpaste Day 7; Fig. 3b).

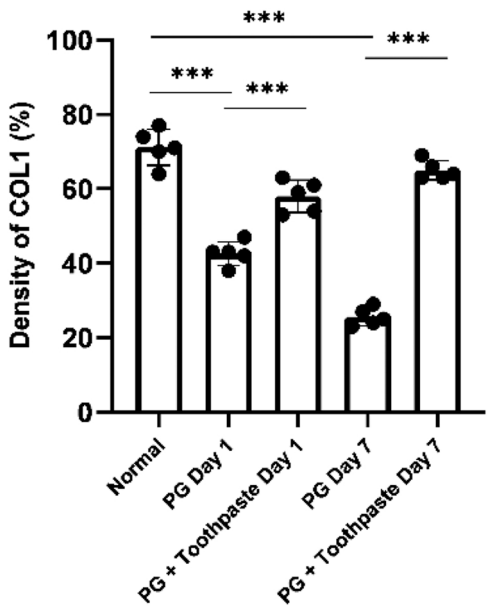

Fig. 2. Density of COL1 (%) based on Masson trichrome staining. Each bar represents the mean ± SD of five rats. ***$p < 0.001$, one-way ANOVA.

The administration of *P. gingivalis* increased the number of MMP1 + and MMP8 + cells in the PG Day 1 and PG Day 7 groups compared with that in the normal group, both qualitatively (Fig. 4a) and quantitatively ($p < 0.001$; Fig. 4b), whereas treatment with *N. sativa* toothpaste decreased the cell percentage at both time points ($p < 0.01$, Normal vs. PG + Toothpaste Day 1, Normal vs. Toothpaste Day 7; Fig. 4a).

4. Discussion

The current study evaluated the effects of *N. sativa toothpaste* in rat model of periodontitis induced by *P. gingivalis*. The current study found that *N. sativa* decreased the number of neutrophils, macrophages, and lymphocytes in periodontal tissue, as well as IL-1β and PGE2 serum concentration. Likewise, the *N. sativa toothpaste* decreased MMP1 + and MMP8 + cells and increased COL1 density in the affected tissue.

N. sativa belongs to the Ranunculaceae family and is a plant with a long history of medicinal use. It is also known as black seed and is often found in Southern Europe, North Africa and Southwest Asia. This plant has a wide range of pharmacological effects, including antihypertensive, analgesic, anti-inflammatory, antibacterial and antioxidant properties (Ahmad et al., 2013). It is widely known for its antibacterial properties. One study reported that 300 mg/mL of *N. sativa* inhibited the growth of *Staphylococcus aureus*, as demonstrated by the presence of a clear zone from the paper disc diffusion method (Bakathir & Abbas, 2011). This antibacterial effect was observed in a clinical study, where the combination of 2 g of *N. sativa* seeds with 40 mg of omeprazole in patients with non-ulcer dyspepsia beneficially eliminated *Helicobacter pylori* when compared with a triple-drug therapy using clarithromycin, amoxicillin and omeprazole (Salem et al., 2010). The amount of thymoquinone, the active component, ranges from 30% to 48% in the *N. sativa* seeds (Ahmad et al., 2013; Mekhemar et al., 2020). Chaieb, Kouidhi, Jrah, Mahdouani, and Bakhrouf (2011) reported that thymoquinone inhibited the formation of biofilms by various bacteria with a minimum inhibitory

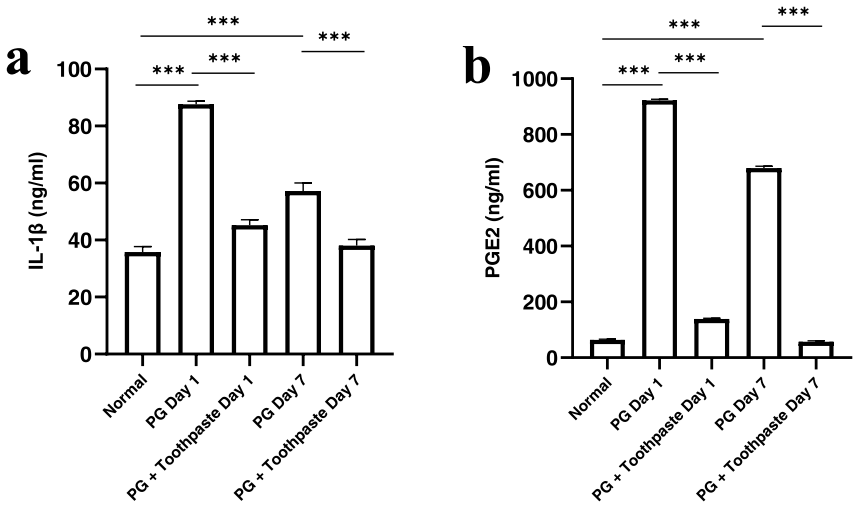

Fig. 3. Levels of IL-1β (a) and PGE2 (b) in rat serum. Each bar represents the mean ± SD in five rats.

concentration ranging from 8 to 32 g/mL. Because of its potent antibacterial activity, *N. sativa* is potentially used as a therapeutic agent or adjuvant in bacterial infections.

Periodontal disease, the most common cause of tooth loss, is a chronic inflammatory disease of the periodontium characterized by the loss of periodontal ligaments and the destruction of surrounding alveolar bone in its advanced stages. Periodontal disease activates inflammatory and immune mechanisms that play a role in the development of systemic disease (Wada & Kamisaki, 2010). Moreover, periodontitis is defined as an inflammatory disease caused by bacteria that destroy the gingiva around the teeth (AlAttas et al., 2016). Inflammation in periodontitis is triggered by organisms in oral microbial biofilms, particularly gram-negative anaerobes such as *P. gingivalis* (Mekhemar et al., 2020). The imbalance between microbial communities resulted in the host's chronic inflammatory response. Induction by *P. gingivalis* linked to the activation of the inflammasome, mainly the NLRP3. Activation of NLRP3 cleaves the pro-form of inflammatory cytokines, such as IL-1β, to the mature form with the help of caspase-1. Activation of caspase-1 also led to the death of osteoprogenitors and osteoclastic bone resorption. *P. gingivalis* activates the NLRP3 inflammasome, which may cause long-term inflammation and promote atherosclerosis and other periodontal diseases (Ding et al., 2020; Rocha et al., 2020).

Other proteins in periodontitis also have been suggested as independent risk factors of cardiovascular diseases (Isola, Polizzi, Alibrandi et al., 2021; Isola et al., 2020). Higher serum and salivary levels of Gal-3 and ADMA were found in periodontitis patients with coronary heart disease (Isola, Polizzi, Alibrandi et al., 2021; Isola et al., 2020). Gal-3 belongs to the β-galactoside-binding lectin family released from inflammatory cells (Velickovic et al., 2021). Moreover, ADMA is an endogenous inhibitor of NO synthase linked to endothelial dysfunction (Okada et al., 2021). This pathological mechanism elevated serum and salivary NLRP in periodontitis patients (Isola, Polizzi, Santonocito et al., 2021), which also positively correlated with caspase-1, IL-1β, IL-18, Gal-3, and ADMA levels (Bostanci et al., 2009; Isola, Polizzi, Alibrandi et al., 2021; Isola, Polizzi, Santonocito et al., 2021; Okada et al., 2021). Because of this, patients with periodontitis have a higher risk of systemic inflammation (Hasturk & Kantarci, 2015).

The periodontitis disease model was successfully conducted in this study. The presence of some humoral and cellular makers could be considered as a primary sign of a successful periodontitis model. Li, Zhang, and Wang (2020) reported an increase in the number of inflammatory cells, particularly neutrophils, in chronic periodontitis tissues compared with that in healthy periodontal tissues. Increases in the levels of cytokines and chemokines, such as immunoglobulin A (IgA), IL-1β, TNF-α, and PGE2, have also been reported in tissues affected by periodontitis (Liao et al., 2014; Rangbulla et al., 2017). In the current study, an increase in the number of neutrophils, macrophages and lymphocytes was observed in the gingival tissue specimens of the rats. The induction of periodontitis following the application of bacteria increased the levels of the inflammatory factors IL-1β and PGE2 in the peripheral blood of the experimental animals, thus indicating the development of innate inflammation in the experimental animals.

Periodontitis is characterized by the presence of gingival tissue destruction (Kawai et al., 2006), which is marked by a decrease in the density of abundant proteins, such as COL1, in the periodontal extracellular matrix (Gursoy et al., 2013). A decrease in COL1 is a result of an increase in the levels of collagenase enzymes, such as MMP8 and MMP1, in the tissue (Kinney et al., 2014; Rangbulla et al., 2017). In the current study, we showed that rats with periodontitis presented with increased numbers of MMP1 + and MMP8 + cells. MMP1 is synthesized by macrophages (Romanelli et al., 1999), whereas MMP8 is a neutrophil-driven collagenase (Gursoy et al., 2013). The increase in MMP1 and MM8 levels in the current study was the result of an increase in the number of neutrophils and macrophages, which led to the damage of the periodontal extracellular matrix resulting in a reduction in the density of the collagen fibers.

The current study showed that *N. sativa* toothpaste treatment can reduce the number of neutrophils, macrophages, lymphocytes, IL-1β and PGE2 levels. Neutrophils, the most abundant white blood cells, are important in the innate immune system. They function as phagocytes to kill extracellular pathogens; similarly, macrophages are phagocytic cells that help promote tissue healing (Scott & Krauss, 2011). Moreover, lymphocytes are responsible for the adaptive immune response (Hajishengallis & Korostoff, 2017). They secrete cytokines and mediators of inflammation, including TNF-α, IL-1β and PGE2, which initiate the inflammation cascade and result in chronic inflammation (Kinney et al., 2014; Liao et al., 2014; Rangbulla et al., 2017). The decreased number of inflammatory cells and mediators in the current study proved that the administration of *N. sativa* toothpaste can treat acute inflammation and prevent chronic inflammation in the animal model of periodontitis.

COL1 is the most abundant protein in the periodontal extracellular matrix (Gursoy et al., 2013; Zhang, Li, Yan, & Huang, 2018). MMP1 and MMP8 are collagenases and members of the MMP family. They are reported to degrade the extracellular matrix in periodontal disease (Romanelli et al., 1999). The current study showed that the administration of *N. sativa* toothpaste increased COL1 density and decreased MMP1 + and MMP8 + cells in rats with periodontitis, thus exhibiting the anti-destructive effects of *N. sativa*.

The anti-inflammatory and anti-destructive activity of *N. sativa* may

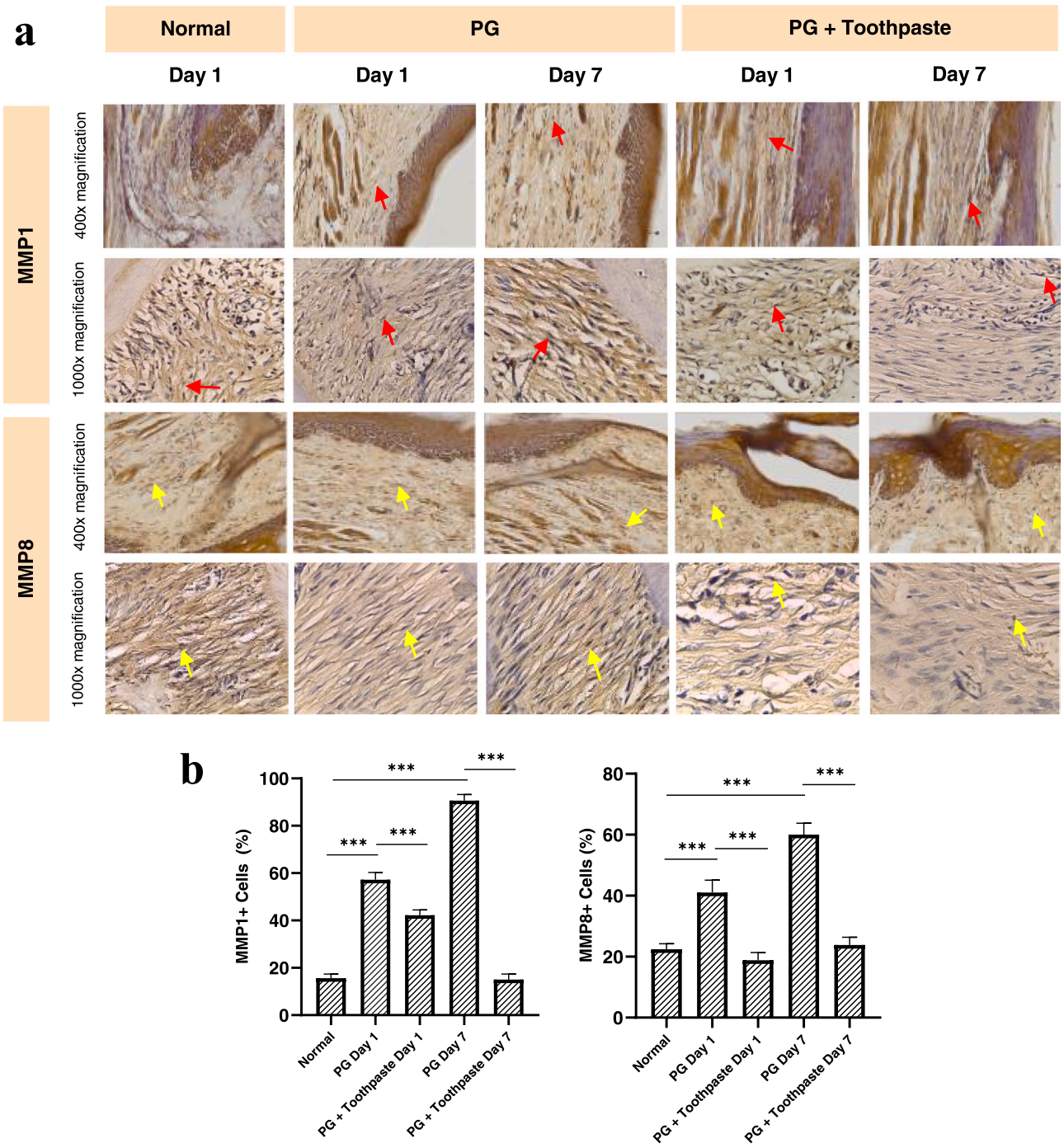

Fig. 4. MMP1 and MMP8 immunohistochemical staining in the gingival tissues (magnification, 400× and 1000x), MMP1 + cells (red arrow), MMP8 + cells (yellow arrow) (a). Percentage of MMP1 and MMP8 immunopositive cells. Each bar represents the mean percentage of immunopositive cells ± SD observed in 10 different visual fields in five rats. ***$p < 0.001$, one-way ANOVA (b).

be due to the presence of the active compound thymoquinone. Thymoquinone shows the major pharmacological actions of *N. sativa,* including anti-inflammatory, antioxidant, antibacterial, analgesic, as well as hypoglycemic, and anticarcinogenic (Ahmad et al., 2013; Chaieb et al., 2011; Mekhemar et al., 2020). Moreover, thymoquinone was reported to reduce several pro-inflammatory cytokines, such as ILs, TNF-α (Shaterzadeh-Yazdi, Noorbakhsh, Hayati, Samarghandian, & Farkhondeh, 2018) and MMP8 (Elamrousy, 2021). Furthermore, thymoquinone demonstrated its antibacterial effect by inhibiting biofilm formation by *Fusobacterium nucleatum* and *P. gingivalis* (Tantivitayakul, Kaypetch, & Muadchiengka, 2020). Thus, taken together, these findings indicate that *N. sativa* possesses antibacterial, anti-inflammatory and anti-destructive properties, particularly in tissues with periodontitis.

5. Conclusions

The effects of using toothpaste containing *N. sativa* on an animal model of periodontitis were examined in this study. The *N. sativa* toothpaste exhibited anti-inflammatory effects by reducing the numbers of neutrophils, macrophages and lymphocytes in the rat tissues. *N. sativa* also reduced inflammatory cell activity by decreasing the levels of IL-1β and PGE2. Furthermore, *N. sativa* toothpaste demonstrated anti-

destructive effects on the periodontal extracellular matrix by decreasing the levels of MMP1 and MMP1 and increasing COL1 density. Thus, *N. sativa* toothpaste can be potentially used as a therapeutic agent or adjuvant for the management of periodontitis. However, further studies in humans are required to confirm these findings.

Funding

This work was supported by the Ministry of Finance of the Republic of Indonesia, through RISPRO research schemes [grant number PRJ-49/LPDP/2019].

CRediT authorship contribution statement

Ernie Maduratna Setiawatie: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing – review & editing. **Maria Apriliani Gani**: Conceptualization, Formal analysis, Software, Validation, Visualization, Roles/Writing – original draft. **Retno Puji Rahayu**: Conceptualization, Formal analysis, Investigation, Methodology. **Noer Ulfah**: Resources, Supervision, Validation. **Shafira Kurnia**: Investigation, Project administration, Validation, Writing – review & editing. **Eka Fitria Augustina**: Investigation, Project administration, Writing – review & editing. **Desi Sandra Sari**: Investigation, Project administration, Writing – review & editing.

Acknowledgements

The authors thank the Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia, for the support during the study.

Declaration of Competing Interest

None.

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