Viability of Nigella sativa Toothpaste with SLS Compared Non-SLS on Fibroblast Cell Culture

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Abstract

Nigella sativa toothpaste with antibacterial, anti-oxidant, and anti-inflammatory properties has beneficial effects in infectious disease, such as gingival inflammation. One of the most widely used synthetic detergents in toothpaste is Sodium lauryl sulphate (SLS). SLS is used to decrease water's surface tension. The side effects of SLS include oral epithelial sloughing, ulcerations, inflammation, protein denaturation, and membrane expansion. This study aimed to determine the viability of human gingival fibroblast (HGFs) cultured with Nigella sativa toothpaste extract containing 2% SLS and Nigella sativa toothpaste without SLS. HGFs were grown in DMEM medium then challenged with Nigella sativa toothpaste with 2% SLS (Nigella sativa-SLS group), Nigella sativa toothpaste without SLS (non-SLS group), and control group. Based on the present study, the cell viability of Nigella sativa-2% SLS, non-SLS, and control group were 92.33%, 96.30%, and 98.99%, respectively. In conclusion, both Nigella sativa with 2% SLS and non-SLS were non-toxic to HGFs. Thus, Nigella sativa toothpaste is potential to treat gingivitis and oral diseases. However, future *in vivo* study for this **is** needed.

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Introduction

The oral cavity is the gateway to all kinds of diseases. It is estimated that around 3.5 billion people in the world are affected by oral cavity diseases. Dental caries and periodontal disease are the most common oral diseases. One of the factors that often causes this disease is poor oral hygiene.[1] Toothbrushing with toothpaste is an inexpensive way to maintain oral health.[2] Several clinical trials found that tooth brushing with toothpaste reduced the number of *Streptococcus mutans* bacteria in human dental plaque,[3] and reduced extrinsic dental stain.[4] In general, toothpaste contains three main components; abrasives, detergents, and herbal extract.

Toothpaste contains abrasives to clean and whiten teeth. Detergents are a surface-active agent ingredient within toothpaste that is known as foaming agent.[5,6] Moreover, herbal extract is used because it has beneficial effects, such as the anti-inflammatory, that inhibited gingivitis and maintains oral health.[7] *Nigella sativa* is a herbal ingredient containing thymoquinone, flavonoid, thymol, and tannins. The addition of *Nigella sativa*, mint, cloves, aniseed, and olive leaf extracts enhances toothpaste activity by providing effective anti-inflammation and anti-oxidant activity.[8] *Nigella sativa* extract inhibited periodontal pathogen such as *Porphyromonas gingivalis* and *Provotella intermedia*. Because of this, *Nigella sativa* is potentially used as adjuvant in periodontal therapy.[9] Our preliminary study found that *Nigella sativa* extract toothpaste with 2% SLS (*Dentomaxxima*) inhibited the growth of supragingival plaque bacteria. *Nigella sativa* extracts by itself also inhibited bacterial plaque.[10]

Sodium lauryl sulphate (SLS) is an anionic surfactant commonly used as detergent ingredient in toothpaste. SLS decreases the surface tension of water and increases

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toothpaste effectiveness in terms of dental plaque removal. SLS, an anionic detergent, binds to positively charged protein side groups, causing a conformational change (denaturation) of the protein.[11] Sodium Lauryl Sulfate 5% has a degenerative effect on the epithelial cell membrane due to the denaturing nature of proteins. The surfactant effect of SLS causes structural changes in the membrane, allowing penetration of the skin. In the acute eye test, SLS 10% caused corneal damage to rabbit eyes if not irrigated. The draize test for products containing SLS 5% causes slight irritation, while products containing 21% detergent cause severe irritation without rinsing. However, various side effects of SLS have been reported.[12-14] Study also found that high concentrations of SLS positively correlated with the incidence of mucosal desquamation. The exposure of SLS on epithelium also induced the incidence of recurrent aphthous ulcers.[15] Thus, this study aimed to determine the viability of human gingival fibroblast (HGFs) after treatment with *Nigella sativa* toothpaste extract containing 2% SLS and *Nigella sativa* toothpaste with no SLS. Through this study, it will be known whether *Nigella sativa* with SLS 2 % interferes with the viability of fibroblast cells or not.

Materials and methods

Human gingival fibroblasts (HGFs) were obtained from Airlangga University, Surabaya, Indonesia. HGFs were isolated from marginal gingival tissues from the interproximal molar papillae of a healthy patient. The patient previously received ethical clearance from the Bioethics Committee of Faculty of Dental Medicine Airlangga University number 661/HRECC.FODM/X/2019. The tissue was minced into two × two × three mm^2 pieces and put in 12-well culture plates (contained DMEM, 100 units/ml penicillin, 100 µg/mL streptomycin, 10% FBS, and 50 µg/mL amphotericin B). The culture condition was 37°C, 5% CO_2, and 95% air. After a week, and every 3 days after that, DMEM was replaced. The cell then delivered from the tissue culture using 0.25% trypsin-EDTA and seeded into 75 cm^2 flasks in the same culture condition with no amphotericin B. For toothpaste treatment, HGFs were cultured in 24-well culture plates (2.5×10^4 cells per well) contained DMEM, 10% FBS, and antibiotics (5% CO_2, 95% air). Phenol red-free medium containing 1.5% FBS was used to replace the culture media for 24 hours.

Exposure of *Nigella sativa* toothpaste to cell culture

The cell culture received one of the following: *Nigella sativa* toothpaste with 2% SLS (*Nigella sativa*-SLS group), *Nigella sativa* toothpaste without SLS (non-SLS group), and cell without toothpaste (control group), all group were cultured in media containing 5% FBS. *Nigella sativa* toothpaste was prepared from a formula containing sodium lauryl sulphate 2% and non-SLS. The *Nigella sativa* toothpaste mixed with demineralized water to a sorbitol solution in beaker glass for 1 min and stirred with a magnetic stirrer. After that, the mixture was weighed, diluted in culture media, and added to the 24-well plates containing HGFs. After 24h, supernatants were harvested, centrifuged for the viability test

MTT assay

MTT assay was performed to determine the viability of toothpaste. The treated 0.5 cm^2 inserts containing HGFs were placed in culture wells containing 0.3 ml (0.5 mg/ml) MTT solution (Sigma-Aldrich St. Louis, MO, USA) and incubated at 37°C for 24 h. For quantitative evaluation of cell viability, 200 ml of extracts were transferred in a 24-well plate, and absorbance was measured at 595 nm by a spectrophotometer (Dynex Technology, Chantilly, Virginia USA). The cell viability was calculated based on:

$$\text{Cell viability} = \frac{(\text{treatment group's absorbance} - \text{media absorbance})}{(\text{cell absorbance} - \text{media absorbance})} \times 100\%$$

Statistical analysis

The significance of each group's cell viability was assessed by one-way ANOVA. The Kolmogorov-Smirnov test was used to determine the normality of data, and Levene's test for the homogeneity of the variances. Moreover, the Bonferoni post hoc test was used to compare the difference of the data. A significance level of $p<0.05$ was used. All statistical tests were performed using SPSS v12.0.

Results

The viability test showed that the viability of all the test groups was above 90%. *Nigella sativa* toothpaste containing SLS (*Nigella sativa*-SLS group) 92,3% and *Nigella sativa* without

SLS 96,3%. The absorbance of fibroblast cell culture and viability is shown in Table 1.

Group	Repilication	Mean absorbance ± SD	Average cell viability (%)
Control	8	0.6918 ± 0.0954	98.99
Nigella sativa tooth paste with 2% SLS	8	0.7182 ± 0.1986	92.33 *
Nigella sativa tooth paste without SLS	8	0.7406 ± 0.1222	96.30

Table 1. The absorbance and viability of *Nigella sativa* toothpaste with SLS and non-SLS.
Note: *significantly difference with the control group.

Table 1 showed that the viability of *Nigella sativa*-SLS group was 92.33%. Moreover, the non-SLS group had 96.3%, while the control group had 98.99% of cell viability. Moreover, a statistical test is carried out to see the significance of each group's cell viability, no significant difference between *Nigella sativa* toothpaste with SLS and non-SLS toothpaste groups ($p>0.05$). However, there is a significant difference between *Nigella sativa* toothpaste containing 2% SLS with the control group ($p<0.05$).

Discussion

Toothbrushing with toothpaste is the most inexpensive way to maintain oral health. Many studies reported the benefits of brushing teeth, such as reduced dentin hypersensitivity,[16] and prevent dental caries.[17] Clinical trials also showed that the use of toothpaste causes a reduction in external dental stain caused by silver diamine fluoride after one and two days of use.[2] Various kinds of toothpaste have been circulating in the market, including those containing herbal ingredients. Herbal is used as toothpaste ingredients because of its beneficial effects, especially anti-inflammatory, anti-oxidant, and antibacterial activity. Nahak *et al.* (2018) reported that toothpaste and mouthwash containing plant extract reduced the number of *Streptococcus mutans* bacteria in human dental plaque.[3] A systematic review and meta-analysis by Janakiram *et al.* (2020) also reported that herbal toothpaste is more effective in reducing dental plaque compared to non-herbal toothpaste.[18]

The present study compared the viability of fibroblast cells treated with *Nigella sativa* toothpaste extract containing 2% SLS with *Nigella sativa* toothpaste without SLS. Human gingival fibroblasts (HGFs) culture was used for the MTT test because fibroblasts are the most abundant periodontium cells and play an essential role in homeostasis and oral cavity pathogenesis. Based on the result, *Nigella sativa* toothpaste containing 2% SLS as well as the non-SLS had viability above 90%. Base on the toxicity parameter of CD 50, *Nigella sativa* tooth paste with 2% SLS categorized as a non-toxic ingredients to fibroblasts.[19]. *Nigella sativa* is a herbs that contain abundant nutrients and phytoconstituents, including thymoquinone, flavonoid, alkaloids, saponins, sterols, and essential oils. This plant has various beneficial effects, including anti-oxidant, antidiabetic, antihypertensive, neuroprotective, anti-inflammatory, analgesic, and antimicrobial activity. Thymoquinone (2-isopropyl-5-methylbenzo-1,4-quinone) is a compound that takes up 30–48% of the *Nigella sativa* seed. Thymoquinone induces an anti-oxidant and anti-inflammatory effect through various free radicals scavenging ability and reduces nitric oxide levels (NO).[20-22] Because of its anti-inflammatory and antimicrobial activity, *Nigella sativa* is potentially used as the toothpaste's main ingredient to treat oral diseases. Masya *et al.* (2019) reported that *Nigella sativa* extract toothpaste with 2% SLS caused 15.1416 ± 0.231 mm inhibition zone on broth containing supragingival plaque *bacteria, and this result was significantly higher than the control group.*[10] *Nigella sativa extract solution provided improvement in gingival inflammation and deterred plaque formation.*[23]

In this study, we used 2% SLS as detergent in the *Nigella sativa* toothpaste formula. SLS is a detergent widely used for toothpaste because it produces foam and increases the effect of a fresh taste sensation. SLS also helps in the dispersion of intra-oral toothpaste and inhibits the growth of several microorganisms. This detergent penetrates through the bacteria cell walls and interacts with cell membrane components, including lipids and proteins. In countries such as America and Europe, many toothpastes contain SLS with a concentration of 0.5% to 2%. Some study reported that SLS might have side effects such as destroying the epithelial cells in the oral cavity when the concentration exceeds 2 %. Penetration of SLS into the membrane increases cell permeability through the oral mucosa's denaturation, leading to intracellular leakage and cell lysis.[24] The

effects of SLS depend on their structure and the components of the targeted cell membrane. 10% SLS increased the release of sulfidryl groups from keratin, which resulted in a 78.9% increase in the liberated sulfidrile groups. Cytotoxicity of SLS is concentration-dependent. However, 2% of SLS did not increase the percentage of sulfidrile groups freed from keratin.[25-26] This study proved that *Nigella sativa* toothpaste containing 2% SLS was non-toxic. Thus, *Nigella sativa* toothpaste with 2% SLS or non SLS is potentially used for daily oral treatment, especially in gingivitis and periodontitis.

Conclusions

Based on the present study, Nigella sativa extract toothpaste with 2% SLS and without SLS are non-toxic to fibroblast cell. The results of the study answered the controversies about the use of SLS for toothpaste. The Nigella sativa extract toothpaste with 2% SLS is potentially used to maintain oral health as well as to treat oral disease. However, further study for this is needed.

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Declaration of Interest

The authors report no conflict of interest.

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