

SODIUM BICARBONATE TOOTHPASTES REDUCE METALLOPROTEINASE-8 AND INTERLEUKIN-1B LEVELS IN SMOKERS AND IMPROVE IMPORTANT SALIVARY INDEXES

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Abstract. Background: Saliva is an important body fluid that aids in digestion and contains a repertoire of important biomarkers. This research aimed to investigate the role of sodium bicarbonate toothpaste in regulating salivary indexes. Materials and Methods: The study involved taking saliva samples from healthy and fasting adult, male volunteers, aged between 20 to 45 years old. The samples were taken randomly and stratified, from 50 smokers and 50 non-smokers. The levels of metalloproteinase-8, interleukin-1 β , pH, salivary flow rate, and α -amylase were measured, and the effects of sodium bicarbonate toothpaste application were investigated. Results: The levels of metalloproteinase-8 and interleukin-1 β in smokers were significantly elevated, with more acidic saliva but reduced salivary flow rate and α -amylase. With the application of sodium bicarbonate, there were significant reductions in metalloproteinase-8 and interleukin-1 β , with substantial improvement in pH, salivary flow rate, and α -amylase in both smokers and non-smokers. Conclusion: This study confirmed the negative effects of smoking. The use of sodium bicarbonate toothpaste enhanced oral health by reducing the pro-inflammatory biomarkers while improving the pH, salivary flow rate, and α -amylase.

Keywords: metalloproteinase-8, interleukin-1 β , salivary indexes, α -amylase, sodium bicarbonate, smoking habit.

List of Abbreviations

SB – sodium bicarbonate

MMP-8 – metalloproteinase-8

IL1 β – interleukin-1 β

SFR – salivary flow rate

α -amylase – alpha-amylase

Introduction

Saliva, a clear, watery, viscous, heterogeneous, and complex biofluid, is an essential oral cavity component, secreted from salivary glands (Ebersole *et al.*, 2024). It is important in maintaining oral health as it contains proteins and peptides that have roles in defence and protection against viruses and germs, maintenance, lubricating food, remineralizing teeth, and for buffering capacity and balancing oral pH (Hassan *et al.*, 2024). There are many salivary biomarkers such as MMP-8 and interleukins, which are vital for immune response, controlling inflammation, boosting antigen representation, and regulating adaptive immunity (Al-Qahtani *et al.*, 2024).

MMP-8 enzyme is produced by granulocytes in saliva in response to collagen damage, particularly in periodontitis, and is an important in-

dicator of periodontitis. Salivary MMP-8 and IL-1 β levels are important indicators of periodontal health, especially in evaluating the impact of smoking (Noh *et al.*, 2022). Smokers with chronic periodontitis tend to exhibit higher salivary MMP-8 and IL-1 β levels. Monitoring salivary MMP8 and IL-1 β could therefore identify individuals at risk of developing periodontitis (Hamza *et al.*, 2020; Do *et al.*, 2023). Smoking also affects pH levels, SFR, and buffering capacity (Beklen *et al.*, 2021). Smokers have shown lower salivary pH levels than non-smokers (Bhavsar, 2023). An inverse correlation between smoking frequency and the effects on salivary pH and flow rate is well-established (Azuma & Matsui, 2022).

One of the effective ways to promote oral health and to moisturize the mouth is to apply SB mouthwash (Mohammadi *et al.*, 2022). SB could increase salivary pH and improve SFR, and IL-1 β levels, especially in smokers (Hamza *et al.*, 2020; Ariani *et al.*, 2023). However, the use of SB toothpaste has not often been promoted for oral health of both smokers and non-smokers and to prevent dental caries especially in children although it is easier to implement.

The current study aimed to investigate the potential role and effects of SB toothpaste in regulating the levels of MMP8, and IL-1 β as pro-inflammatory markers, and in improving the levels of pH, SFR, and α -amylase in the saliva samples of cigarette smokers, in comparison to the non-smokers. The hypothesis of the study is that the SB toothpaste, if used in a regulated manner, could control pro-inflammatory MMP8, and IL-1 β biomarkers and improve pH, SFR, and α -amylase levels in smokers and non-smokers.

Materials and Methods

Saliva samples

The study involved healthy and fasting adult, male volunteers in Basrah, Iraq. The samples were taken randomly and stratified, during the period of 10 months (September 2023 to June 2024), from 50 smokers, and 50 non-smokers, aged between 20 to 45 years old. With a 5% dropout rate, the sample was simply replaced with other sample, provided the volunteers declared that they did not suffer from acute infectious or systemic diseases and were excluded from the study if the inclusion criteria were not met. The sample size was set according to Kish Formula (1965) and ensured not biased. The demographic characteristics (age, height, and weight), as well as the smoking habit and frequency, were recorded. After the screening process, the written approval was obtained from the Research Ethics Committee of the College of Dentistry, University of Basrah (Ref: 15-4/9/2023). All the procedures were performed as per the ethical guidelines laid down by the Declaration of Helsinki (2013).

The participants were refrained from eating, drinking, chewing gum, or smoking for at least 2 h before saliva collection. All subjects underwent an assessment of oral health protocols and instructed to rinse their mouths with water for 15 s to eliminate any microbial debris and epithelial cells. The saliva was collected in the morning at 8-9 am through passive drooling for a specific period to represent accurately the unstimulated whole saliva. The participants were given the SB toothpaste and instructed to use the toothpaste twice daily, after breakfast and

after dinner, for 15 days. They then returned to the lab to give the second saliva sample.

The saliva was collected into sterile tubes to evaluate the levels of MMP-8, IL-1 β , pH, SFR, and alpha-amylase of both smoker and non-smoker groups. In this study, smokers were compared with non-smokers to determine the normal levels of the parameters chosen for the study, which cannot be obtained when comparing smokers with smokers. The researchers, study tools, and procedures can be reproduced based on a pilot study.

Analyses

MMP-8

Unstimulated whole saliva (3 ml) was put in sterile 5 ml tubes (Navazesh, 1993). and was centrifuged ($1000 \times g$ for 10 min at 4 °C). The supernatant was dispensed into Eppendorf tubes and kept at -80 °C until it was transferred to the lab and analysed.

Salivary MMP-8 levels were analysed by Human Total MMP-8 Quantikine immunoassay kit using enzyme-linked immunosorbent assay (ELISA) method, from R and D Systems (Minneapolis, USA). The absorbance was read at 450 nm, at the wavelength of 540 nm.

IL-1 β

The interleukin levels were determined after centrifuging the saliva samples at high speed to get the supernatant. It was then transferred to Eppendorf tubes and kept at -80 °C before analysis. The IL-1 β levels were assessed by utilizing the enzyme-linked immunosorbent (ELISA) method based on the technique of sandwich ELISA (Elabscience, USA). The absorbance was read at 450 nm, and the concentrations of salivary IL-1 β were estimated from the standard curve.

pH

The pH was determined using pH strips. The pre-rinsed mouth of the participant was filled with saliva and then swallowed to remove any acidic bacteria that may be latent. Then, a small amount of saliva was put on a tablespoon and the pH strip was immediately dipped into the sample. When the pads on the test strip were

sufficiently covered in liquid, the strip was removed. After 15 s, the reading of the pH strip gave an index of acidity or alkalinity. A pH between 7 and 7.5 is ideal for saliva, although the saliva tends to be rather acidic.

SFR

The SFR was evaluated by collecting the saliva from each volunteer in a resting state. The quantity of saliva collected was then meticulously quantified and scrutinized. This approach guaranteed that all ethical considerations were addressed and that the data collection process was uniform for all participants. The criteria that was considered during the study included the use of dental braces or dental prostheses, medications that disrupt the salivary flow, allergies to oral hygiene products, dental materials, or therapeutic substances used. This enabled dependable comparisons between SFR before and after applying the SB toothpaste, especially by the non-smokers (Trottier, 2016). The SFR was also calculated based on the saliva amount collected during a specific period.

α -amylase

Starch (0.1 g) was dissolved in 100 ml of distilled water. Then, 5 ml of the starch solution was pipetted into T (Test) and C (Control) test tubes. The T-tube was placed in a water bath at 37 °C for 5 min, while the C-tube was left at room temperature. The T-tube was added with 20 μ l of saliva and 30 μ l of normal saline, while the C-tube was added with 50 μ l of normal saline. Both tubes were then placed in the water bath for about 7.5 min. After the incubation period, both tubes were removed from the water

bath and 5 ml of iodine was immediately added to the T and C-tubes, assigned as group A tubes. Another two test tubes (T and C-tubes) were added with 2 ml of distilled water and assigned as group B tubes. Two ml of group A tube solution was transferred to the group B tube. The absorbance of the sample against the water blank was read at 670 nm wavelength in a spectrophotometer using a cuvette with a 1 cm light path (Hasan, 2017).

Statistical analysis

Statistical study was carried out to obtain the means and standard deviations, and the T-test (dependent and independent) was applied to compare the two groups. The confidence level was set at 95%, so that the value of $P < 0.05$ is considered statistically significantly different. Data analysis was performed using IBM-SPSS statistical program version 22 (IBM Corp, Armonk, NY, USA).

Results

Based on baseline measurements in Table 1, the MMP8 and IL-1 β were significantly higher in smokers than in non-smokers. The salivary pH was more acidic and the SFR was significantly lower in smokers than in non-smokers. However, the α -amylase level was not significantly different. After using the SB toothpaste, there were significant reductions in the salivary MMP8 and IL-1 β levels in the smoker group (Fig. 1a, b), as similarly observed in the non-smoker group (Fig. 1c, d). Figure 2 shows a significant increase in the salivary pH, SFR, and α -amylase in both the smoker and non-smoker group.

Table 1

Base-line measurements of different salivary parameters in smokers and non-smokers

Parameter	Unit	Smoker (n = 50)	Non-smoker (n = 50)	p-value
MMP8	ng/mL	264.62 $\pm$ 6.21	152.91 $\pm$ 5.15	0.000*
IL-1 β	pg/ mL	367.27 $\pm$ 44.01	203.15 $\pm$ 43.58	0.003*
pH		5.78 $\pm$ 0.18	6.94 $\pm$ 0.22	0.006*
SFR	mL/min	0.94 $\pm$ 0.13	1.51 $\pm$ 0.02	0.003*
α -amylase	U/ml	49.43 $\pm$ 15.61	57.87 $\pm$ 0.23	0.000

Note: * $p \leq 0.05$ is significantly different (degree of freedom = 98)

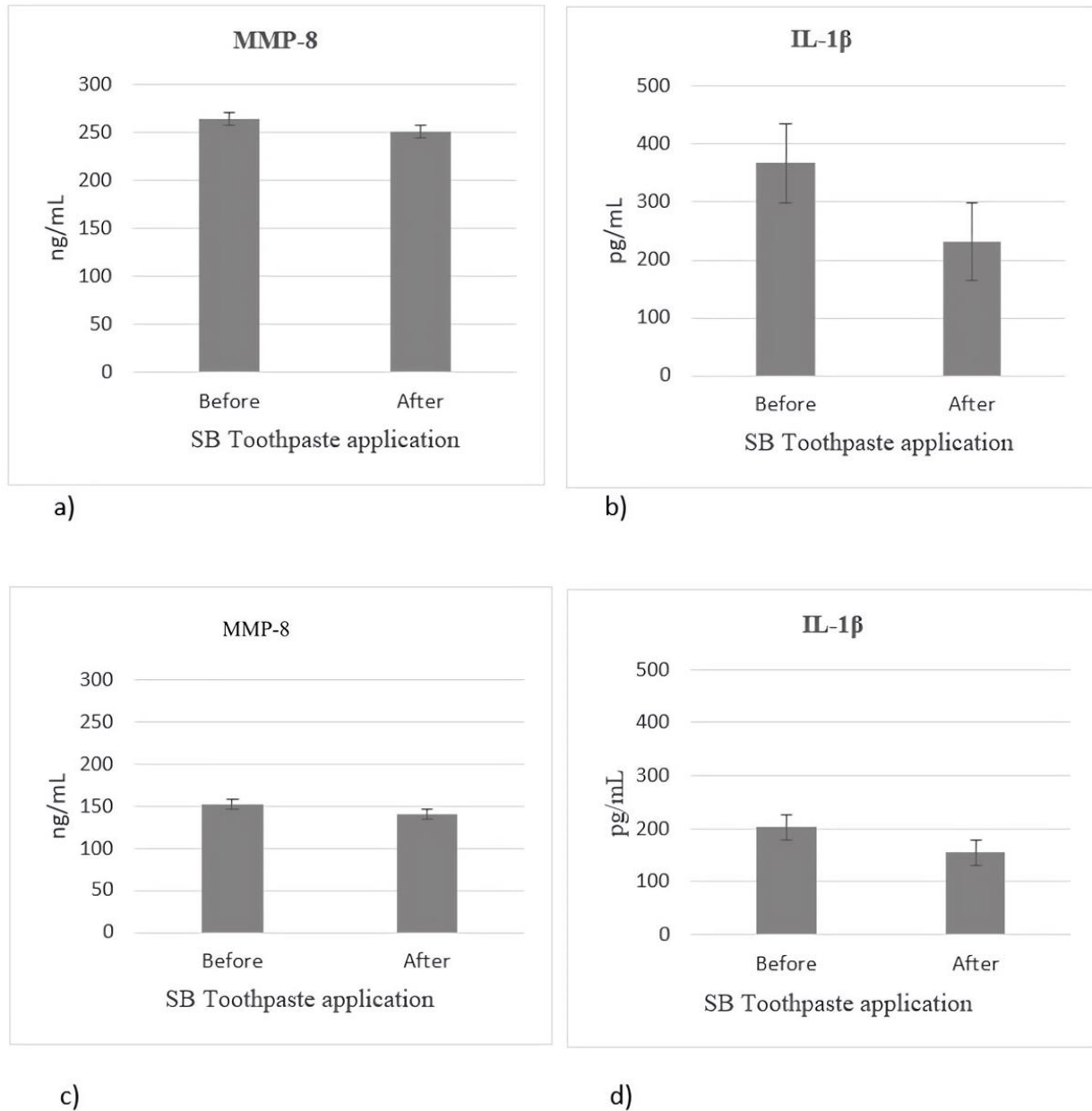


Fig. 1. Comparison of salivary MMP-8 and IL-1β in smokers (a, b), and non-smokers (c, d), before and after using the SB toothpaste

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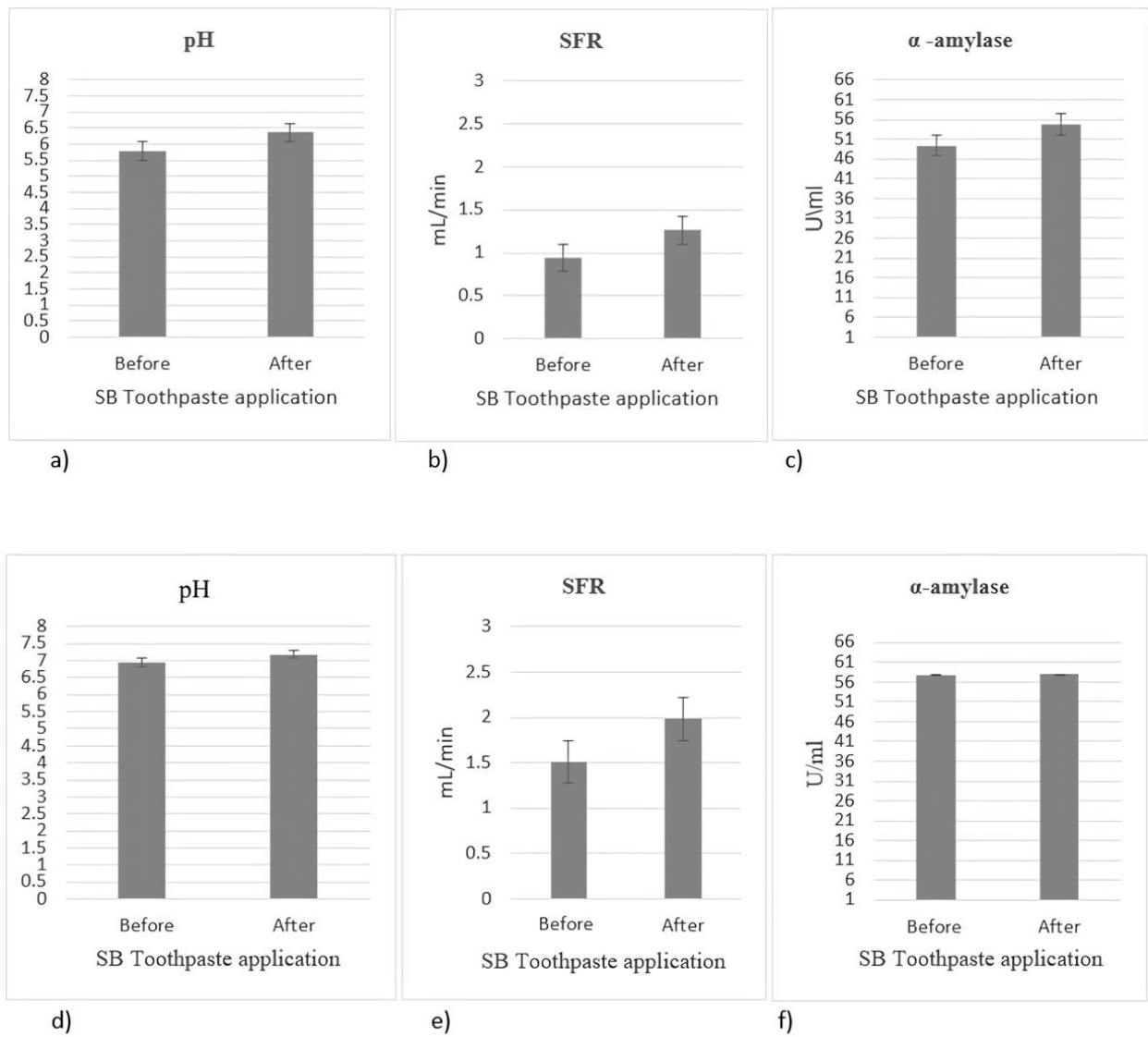


Fig. 2. Salivary pH, FR, and α -amylase levels in smokers (a, b, c), and non-smokers (d, e, f), before and after using the SB toothpaste

Discussion

MMP-8 is the main collagenolytic metalloproteinase in gingival tissues and is the main index in assessing chronic periodontitis. The levels of MMP-8 in periodontitis patients can be associated with smoking habit (Noh *et al.*, 2022). The salivary MMP-8 as a biomarker could determine the status of oral health and serve as an index on the negative impacts of smoking, especially in periodontal disease progression (Do *et al.*, 2023). Our study proved that the MMP-8 and IL-1 β levels were significantly higher in smokers than non-smokers, which are consistent with previous reports (Do *et al.*, 2023; Addissouky *et al.*, 2024). The chemicals in cigarettes may elevate oxidative stress and lower immune responses. The deterioration of periodontal tissues from oxidative stress alters the MMP-8 activity (Luchian *et al.*, 2022). and increases the levels of IL-1 β , TNF- α , and IL-6 in smokers. The inflammation caused by smoking changes the levels of cytokines such as IL-1 β . Interleukins play important roles in controlling inflammation and regulating adaptive immunity (Hamza *et al.*, 2020).

With the SB toothpaste application, the smokers exhibited reduced salivary MMP-8 and IL-1 β levels, indicating positive responses to the therapy. This is clear evidence of the positive effects of SB on important inflammatory salivary biomarkers. The SB could have limited MMP-8 and IL-1 β to greatly reduce the negative impact on oral tissues. The decreased levels after using SB can be due to the regulation of inflammatory responses in the oral cavity. Nicotine may exert immunosuppressive effects in which the SB can effectively resist to create an environment less suitable for inflammation and cytokine release, leading to reduced interleukins in the saliva. The anti-inflammatory properties of SB may exert an important role in modulating the interleukin levels (Hamza *et al.*, 2020).

A significantly lower salivary pH, SFR, and α -amylase in smokers compared to the non-smokers suggests that smoking makes saliva more acidic, affecting negatively the oral environment. Smoking severely disturbs the oral mucosa and harms the oral defence mechanism

(Ahmad & Panchbhai, 2022). Nicotine causes fluctuations in the autonomic nervous system, leading to reduced SFR (Jain *et al.*, 2023). Both the duration and frequency of smoking affect negatively the SFR, which could potentially lead to more serious oral health complications. Decreased SFR and salivary pH from prolonged smoking are early indicators of oral mucosal harm and as non-invasive diagnostic tools to determine pathological changes (Jain *et al.*, 2023). For α -amylase, a decreased level in smokers compared to the non-smokers indicates the impact of smoking on the salivary enzyme activities. This is very much influenced by the duration of smoking and the number of daily cigarettes. Prolonged smoking of about 20 cigarettes or more per day reduces the activity of salivary α -amylase (Bhavsar *et al.*, 2023).

The application of SB toothpaste can improve the oral environment. The interaction with salivary proteins will be much improved for positive implications on the different components and functions of saliva. Use of SB toothpaste enhances saliva secretion and pH, and reduces caries risk (Beklen *et al.*, 2021; Sulastri *et al.*, 2021). The effectiveness of SB lies in reducing inflammatory biomarker levels and increasing salivary pH, both of which are beneficial for protecting the oral environment (Hamza *et al.*, 2020). An increase in the SFR of non-smokers after using SB toothpaste has also been reported (Sulastri *et al.*, 2021) suggesting its broader benefits. Sufficient SFR keeps the mouth moist, and assists in getting rid of food particles, and preventing dental caries and gum disease. The use of SB toothpaste is therefore advantageous for smokers and non-smokers in improving oral health through enhanced SFR and pH levels (Beklen *et al.*, 2021), and reduced inflammatory markers such as MMP-8 and IL-1 β . For smokers, especially, these reduce the negative impacts of smoking on oral tissues (Rasheed *et al.*, 2023). The inclusion of high-pH buffers like NaHCO₃, Na₂CO₃, and CaCO₃ in the toothpaste aids in neutralizing plaque acid and creates a more alkaline oral environment. While previous studies have suggested the benefits of SB mouthwash (Hamza *et al.*,

2020; Ariani *et al.*, 2023), our study has supported the hypothesis and proven that the SB toothpaste application is essential for oral health of both smokers and non-smokers.

Conclusion

This study demonstrated concrete evidence of the positive impacts of SB toothpaste in reducing inflammatory markers like MMP-8 and IL-1 β in saliva, and in enhancing the pH, SFR and α -amylase especially among smokers. The presence of SB in toothpaste could prevent oral diseases and effectively reduce the harmful effects of smoking on oral health. The hidden potential of oral care products containing SB was exhibited in this study to benefit both smokers and non-smokers alike, and also for children as it is easier to implement.

Clinical Significance

Smokers who suffer chronic periodontitis or are at risk of developing periodontitis have higher levels of salivary MMP-8 and IL-1 β . Smoking also affects salivary pH level and flow rate. Sodium bicarbonate mouthwash improves salivary pH, and flow rate, and decreases IL-1 β . In this study, sodium bicarbonate toothpaste was evaluated instead of mouthwash and proven to reduce salivary MMP-8 and IL-1 β levels. Sodium bicarbonate toothpaste also improved salivary pH, flow rate, and α -amylase and should be promoted for oral health of both smokers and non-smokers, and easier to implement for children.

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