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SODIUM FLUORIDE INDUCES OXIDATIVE STRESS IN ORAL BACTERIA BY ALTERING GLUTATHIONE (GSH) AND GLUTATHIONE S-TRANSFERASE (GST) ACTIVITY

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ABSTRACT: Fluoride is present in almost all oral hygiene products with an average concentration range of 250 to 1000 ppm. Fluoride anions have unique biochemical properties and can inhibit a wide variety of metabolic processes. Here we report fluoride toxicity in *Enterococcus faecalis* which is commonly found in oral infections in humans. We grew *E. faecalis* in Luria Bertani medium at different concentrations of NaF (0, 250, 500, 750, and 1000 ppm). The results showed that *E. faecalis* growth was slightly decreased with increases in the concentration of NaF. The total protein level in the treated bacterial cells was slightly changed with 250, 500, and 750 ppm of NaF while with 1000 ppm of NaF there was a significant decrease ($p<0.05$). The glutathione S-transferase (GST) level showed some decrease with 250, 500, and 750 ppm and was significantly decreased ($p<0.05$) with 1000 ppm of NaF. The glutathione (GSH) level fluctuated with increasing concentrations of NaF and was significantly decreased ($p<0.05$) with 250 and 1000 ppm of NaF. Our observations suggest that fluoride can control the growth of pathogenic bacteria in the mouth and can induce oxidative stress in bacterial cells via GSH metabolism.

Keywords: *E. faecalis*; Glutathione; Glutathione S-transferase; Growth curve; Sodium fluoride.

INTRODUCTION

Fluoride has been incorporated into almost all oral hygiene products due to its therapeutic anti-caries effects.[1,2] Fluoride-containing compounds present in toothpastes, like sodium fluoride (NaF), have been proven to prevent tooth caries for individuals of all ages.[3-5] The topical use of fluoride in dental care products helps in the strengthening of teeth by remineralizing tooth enamel in all age groups. However, due to the risk of fluorosis, it concentration in toothpastes is limited to between 250 to 1000 ppm.[6-9] At present, fluoride-containing toothpastes and mouthwashes are available in almost all countries and stand among the most widely recommended products due to their anticariogenic properties.[10-12] The accepted standard concentration of NaF in oral hygiene products is between 500 and 1,500 ppm, as recommended by the WHO.[8-9] Fluoride-containing mouthwashes with 500 ppm of NaF and toothpastes with 1,500 ppm of NaF are also prescribed for the patients with dental caries.[13] Fluoride can reduce the growth of oral pathogenic microorganisms due to its antibacterial and antifungal properties. However, the mechanism of fluoride's action of inhibiting the growth of bacteria is not fully understood.[14,15] Fluoride can inhibit enzyme activity and acid production in oral bacterial within dental plaque.[16] *Enterococcus faecalis,* a Gram-positive bacterial strain, is commonly found in many human oral infections such as caries, endodontic infections, periodontitis, and peri-implantitis.[17-18]

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GSH plays a significant role as the main antioxidant defense in bacteria and helps in maintaining the intracellular reducing environment.[19] The presence of GSTs in bacteria was first reported by Shishido in 1981.[20] GST enzymes are involved in detoxification and regulate the redox balance under oxidative stress conditions. GSTs and GSH are found in both eukaryotic and prokaryotic cells and play a key role in cellular detoxification in bacterial cells.[21-23]

The aim of the present work was to investigate the mechanisms of toxicity associated with the exposure of *E. faecalis* to NaF within the same concentration range present in oral hygiene products, like tooth pastes and mouth washes. Specifically, we addressed the impact of fluoride on the growth curve, and the levels of total protein, glutathione S-transferase (GST), and glutathione (GSH).

MATERIAL AND METHODS

Bacterial culture and growth conditions: Enterococcus faecalis, a Gram-positive bacterium was obtained from the central laboratory of King Saud University. *E. faecalis* cells were cultured at 37ºC in Luria Bertani (LB) media overnight with shaking at 130 rpm.

Growth curves of the bacteria: To explore the influence of fluoride on the *E. faecalis* growth rate, NaF was adjusted to final concentrations of 0, 250, 500, 750, and 1000 ppm in the LB medium. The growth trends were monitored at each 60 min interval by measuring the optical density (OD) at 600 nm.

Response of the antioxidants and the total protein in the bacteria exposed to NaF: The *E. faecalis* cells were incubated with NaF, adjusted to 0, 250, 500, 750, and 1000 ppm, in LB medium for 24 hr and then harvested.

Preparation of crude extracts: After harvesting, the cells were centrifuged at 12,000 g for 15 min. The cells were washed twice with ice-cold normal saline solution before lysing. The cells were then sonicated for 99 rounds in ice-cold normal saline for 3 sec. Finally, the crude extract was centrifuged at 15,000 for 10 min and the supernatant collected in a sterile tube for later use in assays.

Quantification of the total protein, GSH, and GSTs: The protein was estimated by the Bradford method using bovine serum albumin as a standard.[24] The GSH was assayed by the method of Beutler et al.[25] The activity of the GSTs was assessed using an assay kit from Biovision, USA.[26]

Data analyses: Data were expressed as mean±SD of three replicates.

RESULTS

Growth curves of bacteria: The results of the growth curve of *E. faecalis* in the presence 0, 250, 500, 750, 1000 ppm of NaF in LB medium are shown in Figure 1. The results clearly show that the growth curve is deviated with increased levels of NaF in a concentration-dependent manner.

The response of the total protein in E. faecalis exposed to NaF: As shown in Figure 2, the total protein level of *E. faecalis* was significantly decreased ($p<0.05$) with 1000 ppm of NaF as compared with the control. A slight increase of the protein level was observed with 250 and 500 ppm NaF as compared with the control.

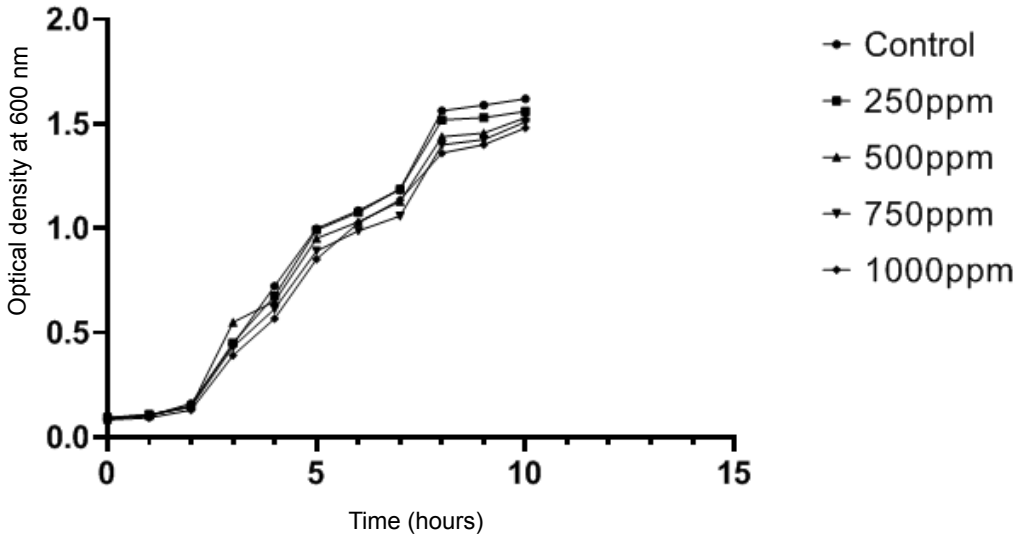

Figure 1. Growth curves of *E. faecalis* incubated with different concentrations of NaF.

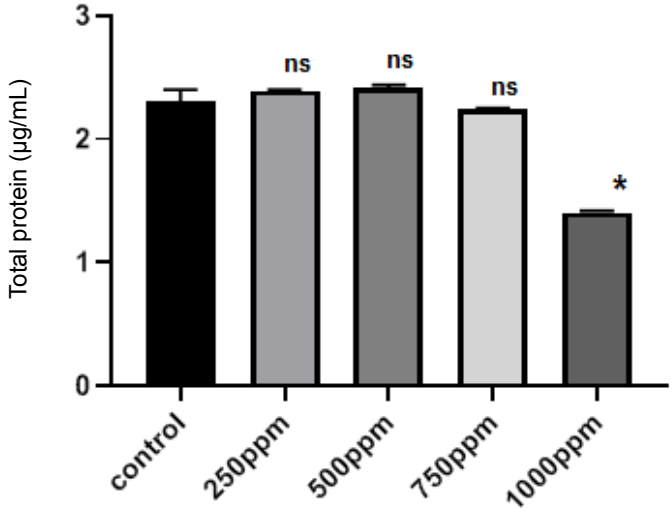

Figure 2. Change in total protein in *E. faecalis* incubated with different concentrations of NaF for 24 hours.
*p<0.05. Values with asterisks are significantly different from the control group.
ns = Non-significant when compared to the control group

The response of the GSH in E. faecalis exposed to NaF: As shown in Figure 3, there was a fluctuation in the GSH level with increasing concentrations of NaF. As compared with the control group, the GSH level was significantly decreased ($p<0.05$) with 250 and 1000 ppm of NaF and showed a non-significant increase with 500 ppm NaF.

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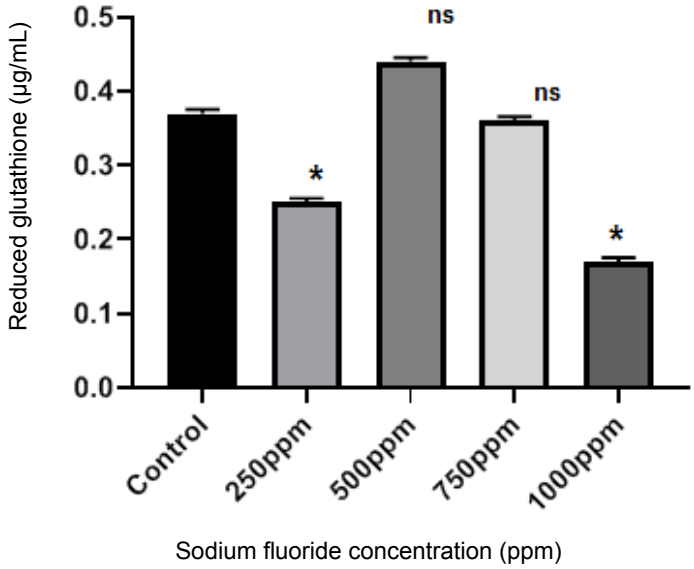

Figure 3. Change in the GSH level in *E. faecalis* incubated with different concentrations of NaF for 24 hours.
*p<0.05. Values with asterisks are significantly different from the control group.
ns = Non-significant when compared to the control group

The response of the GSTs in E. faecalis exposed to NaF: As shown in Figure 4, compared to the control group, the activity of the GSTs decreased in a concentration-dependent manner with increases in the concentration of NaF, with the decrease at 1000 ppm of NaF being significant ($p<0.05$).

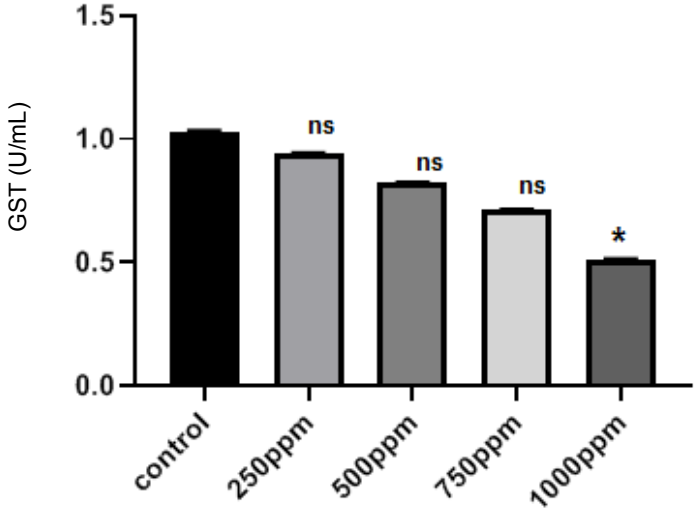

Figure 4. Change in GST activity in *E. faecalis* incubated with different concentrations of NaF for 24 hours.
*p<0.05. Values with asterisks are significantly different from the control group.
ns = Non-significant when compared to the control group

DISCUSSION

Fluoride is the smallest and the most electronegative anion in the halides group with unique biochemical properties. Although it is well known that some bacteria and fungi cannot withstand long exposure to fluoride,[27] the mechanism by which these microbes respond to fluoride is not fully understood. *E. faecalis* is considered to be one of the 25 most abundant pathogens causing persistent endodontic infections and enterococci are present in 3.7–35% of patients with gingivitis or peridontitis.[18] Sixty % of diabetic patients yielded oral *E. faecalis* and *E. faecum*, as opposed to only 6.6% in the controls.[18]

The experimental approach of the present study was to evaluate the effects of fluoride on the growth and antioxidant defense of *E. faecalis*. The concentration of fluoride was fixed according to what is prescribed in mouthwashes and toothpastes. As shown in Figure 1, NaF slightly inhibited the growth of *E. faecalis* with increasing concentrations. The observed growth trends indicate that the fluoride present in toothpastes and mouthwashes can control the pathogenic bacterial growth in the mouth. Our results are in agreement with many previous studies which show that fluoride can inhibit bacteria growth.[16,28,29] Exposure to NaF for one day decreased the total protein concentration and the GST activity (Figures 2–4). The GSH levels also fluctuated with exposure to NaF.

One of the major modes of detoxification in bacterial cells is through the consumption of GSH via a GST-catalyzed reaction. In the current study, we found that NaF is likely to impair this potent protection mechanism by decreasing the GST activity. The decrease in total protein concentration demonstrated the presence of NaF-induced oxidative stress in the test bacteria, since cellular protein concentrations are usually imbalanced under such conditions due to proteolysis. Strong oxidative stress in cells can induce proteolysis resulting in a decrease in the overall protein concentration.[30] The main function of reduced glutathione (GSH) is to keep the redox state in equilibrium in the cytoplasm of a cell. We found a significant decrease in the GSH content with 250 and 1000 ppm NaF which may be due to the environmental stress of the fluoride anion. In a previous study, Hultberg[31] showed that a decrease in the GSH content of bacterial cells may occur with exposure to cadmium and noted that the GSH can be a marker of the intensity of environmental stress in bacteria. On the other hand, our finding of non-significant increases in the GSH concentrations with 500 and 750 ppm of NaF suggests a direct participation of GSH in reactive oxygen species (ROS) detoxification. In both cases, GSH contributes to the protection against oxidative stress in bacteria.[32,33]

CONCLUSIONS

Our data indicate that the fluoride present in toothpastes and mouth washes may play a major role in controlling the growth of pathogenic bacteria in the mouth. Sodium fluoride can induce oxidative stress in bacterial cells by creating fluctuations in the protein concentration and the GSH metabolism.

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