

# Original Research Article

## Impact of ginger-containing dentifrice on enamel surface

### ABSTRACT

**Aim:** Ginger has demonstrated an antiemetic effect and may serve as an alternative for preventing nausea and vomiting during pregnancy (NVP). Incorporating ginger into a dentifrice could offer therapeutic benefits for managing NVP. This study aimed to evaluate an experimental dentifrice containing ginger with respect to its impact on cell viability and potential effects on tooth enamel. **Study Design:** The ginger-containing dentifrice (GD) was formulated, and Colgate Total® 12 served as the control group (CG) to perform cell viability, flow, surface roughness and colorimetry tests. **Place and Duration of Study:** Faculty of Health Sciences of University of Brasilia and School of Dentistry of Federal University of Minas Gerais, Brazil. Between November 2022 and September 2024. **Methodology:** Cell viability following direct contact with the experimental dentifrice (n=2) was assessed using the MTT cytotoxicity assay, in accordance with ISO 10993-5. The flow test was performed following ISO 6876 guidelines (n=3). Using a brushing simulator, 20 bovine incisors (n=10 per group) were coated with 10 mL of dentifrice and 30 mL of water (1:3 ratio) and subjected to 10,000 brushing cycles. The enamel surfaces were analyzed before and after brushing to assess surface roughness (Ra, Rz, and Rq) and colorimetry parameters ( $\Delta E$ ,  $\Delta L$ ,  $\Delta A$ ,  $\Delta B$ , and  $\Delta E_{2000}$ ). Data were analyzed using the t-test and Mann-Whitney test at a significance level of 5%, employing appropriate statistical software. The ginger dentifrice preserved cell viability after 24 hours of direct contact. **Results:** The GD demonstrated a flow of  $51.8 \pm 0.6$  mm, significantly higher than that of the CG ( $45.8 \pm 0.6$  mm). After brushing, no significant differences were observed between groups in final roughness values. Similarly, colorimetric analysis revealed no significant differences in  $\Delta E$  ( $p=0.25$ ) between the groups. **Conclusion:** The GD did not induce cytotoxic effects, nor did it alter the roughness or color of tooth enamel.

**Keywords:** Toothpastes. Hyperemesis Gravidarum. *Zingiber officinale*

### 1. INTRODUCTION

Pregnancy is a phenomenon characterized by physical, social, psychological, and behavioral changes that prepare a woman's body for fetal development, childbirth, and breastfeeding [1]. Nausea and vomiting are among the most common symptoms during this period, affecting 50% to 80% of pregnant women [1,2]. Most women with nausea and vomiting of pregnancy (NVP) experience symptoms limited to the first trimester, while a small percentage may have a prolonged course with symptoms extending until delivery. In such cases, the woman may develop hyperemesis gravidarum (HP), a pathological form of NVP that, if left untreated, can lead to maternal and fetal morbidity [1,3]. Various factors have been associated with NVP and HG; however, their exact pathogenesis remains largely unclear [3]. In this context, the management and prevention of NVP may involve

modifications in eating habits, nutritional counseling, emotional support, acupuncture, medication, vitamins, and other interventions [1,2]. However, due to concerns about teratogenic effects, pregnant women are often reluctant to use medications [3,4].

To avoid the use of certain drugs and substances during pregnancy, there is an increasing trend towards natural alternatives [5,6]. In this context, some herbs have been studied for their potential in preventing NVP, including ginger, chamomile, pomegranate, and cardamom [5]. The use of ginger is a non-pharmacological intervention recommended by the American College of Obstetrics and Gynecology to help control NVP. Ginger, a plant from the Zingiberaceae family, is originally native to Asia and is now cultivated in tropical regions, including Brazil [7]. Ginger products exert an antiemetic effect through several mechanisms, including gingerol and shogaol, which reduce gastric contractions while increasing gastrointestinal activity and exhibiting anti-serotonin effects [5,8,9]. Regarding the safety of internal ginger use during pregnancy, previous studies, including systematic reviews, have classified this root as low risk, making it one of the plants with the lowest risk for use during pregnancy [7,9,10,11].

Regarding oral care and pregnancy, it is known that they influence each other. Increased hormone levels can make the oral cavity more susceptible to dental problems caused by bacteria and plaque [12]. Several studies have found evidence linking poor maternal oral health with adverse pregnancy outcomes and dental health issues in offspring. These outcomes may include preterm delivery, low birth weight, and an increased risk of early caries in infants [12,13]. Dental hygiene itself can predispose pregnant women to nausea and vomiting, as changes in olfaction and taste may lead to intolerance to the taste and smell of oral hygiene products, potentially increasing the risk of dental caries [13]. Dentifrices, contain various substances that serve functions such as polishing, coloring, foaming, humectancy, binding, sweetening, flavoring, and providing fluoride as a therapeutic agent [14,15]. Conventional formulations often include substantial amounts of aromatic oils, which impart flavors such as mint, cinnamon, and eucalyptus [12,13].

In this context, considering the importance of oral hygiene for both maternal and fetal health during pregnancy, and the growing interest in natural products to mitigate NVP [5,6], the incorporation of ginger into a dentifrice has a possible antiemetic and therapeutic effect. Previous studies have shown that ginger extract possesses substantial antimicrobial and anti-inflammatory properties, with potential for prevention of gingivitis and dental caries [16,17,18,19,20]. Ginger essential oil also presented bioadhesive characteristics, providing a prolonged effect [20]. However, at this point, there are no previous studies testing the effects of an experimental dentifrice containing ginger on enamel.

Therefore, the aim of this study was to evaluate an experimental dentifrice containing ginger regarding its impact on cell viability and potential effects on tooth enamel, including color change and abrasiveness.

## **2. MATERIAL AND METHODS**

### **2.1 Ginger Dentifrice Formulation**

The ginger dentifrice (GD) was formulated with the following components: gum arabic (1g), Nipagin (0.2g, methylparaben), calcium carbonate (5g), sodium lauryl sulfate (0.5g), glycerin (1.2g), Natrosol (1g, hydroxyethylcellulose), propylene glycol (0.2g), sodium fluoride (2g), alcohol (1g), and ginger powder (0.7g). The components were measured using an analytical balance (AUW220D, Shimadzu, Kyoto, Japan) and mixed with a magnetic stirrer (IKA C-MAG S7) at 100 RPM (revolutions per minute). After homogenizing the

mixture, it was heated to 40 °C for 30 seconds. The resulting samples were stored in 30 mL plastic tubes.

## **2.2 Cytotoxicity Assessment**

Cytotoxicity was evaluated according to ISO 10993-5:2009 using fibroblasts. The assay was performed in 96-well plates, where  $8 \times 10^4$  cells were cultured with samples placed on stickers (3 mm in diameter and 1 mm in thickness) containing the experimental dentifrice (n=2). The medium alone was used as negative control. The cell culture was maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum. The plates with cells and samples were incubated at 37 °C for 24 hours. Following this incubation, 150 µL of 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT) solution was added to each well, and the plates were incubated again at 37 °C for 3 hours. Subsequently, 100 µL of dimethyl sulfoxide (DMSO) was added to solubilize the formazan crystals formed from MTT. The formation of formazan crystals indicates cell viability, as it reflects the accumulation of MTT in cells via endocytosis, a key characteristic of living cells. The analysis was carried out by spectrophotometry at a wavelength of 540 nm.

## **2.3 Flow Test**

Colgate Total® 12 (Colgate-Palmolive Company, New York, USA) dentifrice was used as the control group (CG) for all the tests. The flow of the dentifrice was assessed using the spreadability method, adapting Specification number 57 of the American Dental Association (ADA). A volume of 0.5 mL of each dentifrice (n=3) was deposited with a 3.0 mL Luer syringe at the center of a glass plate measuring 10 cm by 10 cm. A set consisting of another glass plate and an additional weight totaling 120 g was then placed on top of the dentifrice for 1 second. After removing the additional weight, the largest and smallest diameters of the resulting discs were measured using a digital caliper (Mitutoyo Sul Americana, Suzano, São Paulo, Brazil). For the test to be valid, two conditions were required: the difference between the vertical and horizontal diameters of the discs could not exceed 1 mm, and the disc had to be uniformly circular. The test was conducted in triplicate for each group, and the arithmetic mean of these measurements was used to represent the flow of each group.

## **2.4 Brushing Simulation**

The samples were prepared using PVC pipes measuring 25 × 20 mm as supports. Twenty bovine incisors crowns (n=10) were then embedded in the pipes using acrylic autopolymerizable resin, leaving the vestibular enamel surface exposed. The specimens were polished using a polishing machine (Politriz Lixadeira Metalográfica PVV, Teclago, Vargem Grande Paulista, São Paulo, Brazil). Aluminum oxide discs with sequential grit sizes of 400, 600, and 1000 were used for the polishing process.

Slurries were prepared by mixing 30 mL of distilled water with 10 mL of each dentifrice (in a 1:3 ratio) and were mixed using a magnetic stirrer for 5 minutes. Soft brushes (Medfio®, Medfio Dental Articles Industry and Trade Ltd., Pinhais, PR, Brazil) with nylon

bristles, comprising 34 separate tufts, were used for the brushing process. For the test, the samples (n=10) were placed on a toothbrushing simulation machine (Toothbrushing Simulator Machine 4, Odeme, Luzerna, Santa Catarina, Brazil). A total of 10,000 brushing cycles was performed.

## 2.5 Surface Roughness

A surface roughness tester (Mitutoyo SJ-210®, Mitutoyo Sul Americana, Suzano, São Paulo, Brazil) was used, equipped with an LED display, a diamond point stylus, and a reference standard for roughness measurements (Ra, Rz, and Rq). The test followed ISO standards from 1997. Two readings (along the x and y axes) were taken, spaced 1 to 2 mm apart on each of the flatter samples. The tester was loaded and moved across the tooth surface at a speed of 0.25 mm/second. Measurements were recorded before and after the brushing test.

## 2.6 Colorimetric Analysis

A portable spectrophotometer (Vita Easyshade®, Vita Zahnfabrik h. Rauter GmbH and Co, Germany) was used to quantify the color differences, including  $\Delta E$ ,  $\Delta L$ ,  $\Delta A$ ,  $\Delta B$ , and  $\Delta E_{2000}$ , based on the colorimetric relationship recommended by the C.I.E. (Commission Internationale de l'Éclairage). Color changes were analyzed after the brushing periods and compared to the initial measurements.

## 2.7 Statistical Analysis

The data obtained from the cytotoxicity, flow, roughness, and color stability tests were analyzed using the t-test. A paired t-test was employed to compare the initial and final roughness within the same group. The Mann-Whitney test was used to compare final roughness between different groups when the results presented a non-parametric distribution. All analyses were conducted with a significance level set at 5%, using appropriate statistical software (The Jamovi Project, 2024, version 2.5).

## 3. RESULTS

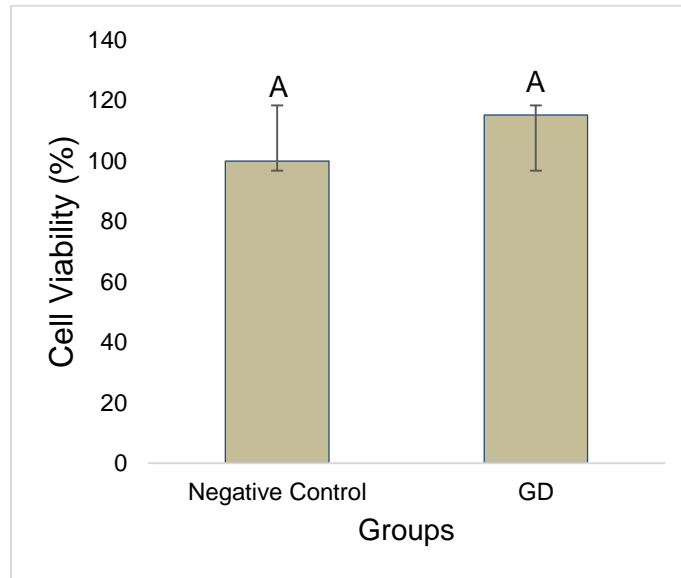
The ginger dentifrice did not cause a decrease in cell viability after 24 hours of direct contact (Figure 1). No statistical difference in cell viability was observed between the negative control and the experimental dentifrice at 24 hours ( $P = 0.22$ ).

The flow measurements for each group (GD and CG) are shown in table 1. GD had a higher flow than CG ( $p < 0.001$ ).

The surface roughness data for the x-axis and y-axis are detailed in Tables 2 and 3, respectively. No statistical differences were found between the groups for the final roughness measurements on the x-axis and y-axis. However, when comparing initial and final roughness on the y-axis, CG showed an increase in roughness after brushing for the parameters Ra, Rq, and Rz.

The colorimetric results for both groups are described in table 4. No significant differences were found between the groups for the different color parameters ( $P > .05$ ).

**Figure 1.** Cell viability (%) of the ginger dentifrice (GD) after 24 hours of direct contact. Equal capital letters indicate no statistical difference in the same column.



**Table 1.** Mean and standard deviation of the diameters (in mm) of dentifrice flow for ginger dentifrice (GD) and control group (CG).

Group	Flow (mm)
GD	51.8 ± 0.60 <sup>A</sup>
CG	45.8 ± 0.60 <sup>B</sup>

Different capital letters indicate statistical differences within the same column ( $P < .05$ ).

**Table 2.** Mean and standard deviation of roughness along the x-axis: transverse to the long axis of the tooth.

Parameter	Group	Initial	<i>p</i> value	Final	<i>p</i> value	<i>p</i> value
		Mean ± SD		Mean ± SD		
Ra	GD	0.18 ± 0.08 <sup>Aa</sup>	0.83 <sup>*</sup>	0.17 ± 0.05 <sup>Aa</sup>	0.96 <sup>#</sup>	0.45 <sup>*</sup>
	CG	0.17 ± 0.06 <sup>Aa</sup>		0.15 ± 0.06 <sup>Aa</sup>	0.56 <sup>#</sup>	

Rq	GD	$0.20 \pm 0.09^{Aa}$	0.88*	$0.23 \pm 0.06^{Aa}$	$0.23^{\#}$	0.47*
	CG	$0.20 \pm 0.07^{Aa}$		$0.20 \pm 0.08^{Aa}$	$0.98^{\#}$	
Rz	GD	$0.95 \pm 0.42^{Aa}$	0.72*	$1.14 \pm 0.36^{Aa}$	$0.12^{\#}$	0.35*
	CG	$1.01 \pm 0.32^{Aa}$		$0.99 \pm 0.32^{Aa}$	$0.81^{\#}$	

Different capital letters indicate statistical difference within the same column (P<0.05).

Different lowercase letter indicates statistical difference within the same line (P<0.05).

Symbols denote the statistical tests used (\*t-test; #Paired t-test)

**Table 3.** Mean and standard deviation of roughness along the y-axis: parallel to the long axis of the tooth.

Parameter	Group	Initial	p value	Final	p value	p value
		Mean $\pm$ SD		Mean $\pm$ SD		
Ra	GD	$0.14 \pm 0.03^{Aa}$	0.02**	$0.18 \pm 0.08^{Aa}$	$0.12^{\#}$	0.58**
	CG	$0.11 \pm 0.04^{Ba}$		$0.17 \pm 0.09^{Ab}$	$0.004^{\#}$	
Rq	GD	$0.18 \pm 0.04^{Aa}$	0.04**	$0.23 \pm 0.09^{Aa}$	$0.12^{\#}$	0.28**
	CG	$0.14 \pm 0.05^{Ba}$		$0.18 \pm 0.08^{Ab}$	$0.05^{\#}$	
Rz	GD	$0.90 \pm 0.20^{Aa}$	0.05*	$1.07 \pm 0.42^{Aa}$	$0.13^{\#}$	0.39*
	CG	$0.69 \pm 0.25^{Aa}$		$0.91 \pm 0.38^{Ab}$	$0.05^{\#}$	

Different capital letters indicate statistical difference within the same column (P<0.05).

Different lowercase letter indicates statistical difference within the same line (P<0.05).

Symbols denote the statistical tests used (\*t-test; #Paired t-test; \*\*Mann-Whitney test)

**Table 4.** Mean and standard deviation of colorimetric measurements.

Group	$\Delta L$	$\Delta a$	$\Delta b$	$\Delta E$	$\Delta E_{2000}$
GD	$-0.66 \pm 2.58^A$	$-0.20 \pm 0.83^A$	$-2.31 \pm 1.59^A$	$3.87 \pm 1.72^A$	$2.03 \pm 1.06^A$
CG	$1.23 \pm 2.05^A$	$-0.85 \pm 0.58^A$	$-2.84 \pm 1.48^A$	$4.84 \pm 1.97^A$	$2.70 \pm 1.28^A$

Equal capital letters indicate no statistical difference within the column ( $P > 0.05$ ).

\*t-test

#### 4. DISCUSSION

The antiemetic effects of ginger have been well-established in several studies, and its safe use during pregnancy has also been tested [7,9,10,11,21]. This is the first study testing the local effects of a dentifrice with the goal of using it to alleviate nausea and vomiting during pregnancy without causing harm. The dentifrice containing ginger was found to have no detrimental effects on cell viability or the enamel surface.

Incorporate ginger into a dentifrice while maintaining the fundamental properties of dentifrices is essential for its effectiveness. Each component plays a crucial role in ensuring that the formulation achieves the desired cosmetic and preventive-therapeutic effects [22]. Dentifrices usually contain excipients and active principles [22]. Excipients include gelling or binding agents, abrasives, surfactants, humectants, colorants, and flavors [22,23]. As a gelling agent, GD used hydroxyethyl cellulose, while CG uses carrageenan. GD contains calcium carbonate as an abrasive, while CG contains silica. Propylene glycol was used as a humectant in GD, while CG contains glycerol. Both dentifrices contain sodium lauryl sulfate as a surfactant. CG used titanium dioxide as colorant. No colorants or flavors were added to GD, except for the natural flavor and color derived from ginger.

Knowing the composition is essential for analyzing the properties of resultant dentifrices. Regarding cytotoxicity, previous studies have shown that ginger extract exhibits minimal cytotoxicity to human gingival fibroblasts, even at the highest tested concentration [24,25]. The present study confirms the safety and potential of using ginger as a natural alternative into a dentifrice. This is especially important when considering the clinical indication for its use during pregnancy to control episodes of nausea and vomiting. In this context, oral care during pregnancy can be carried out without issues.

In this same context, flow is an important property that ensures the product reaches areas that the toothbrush may not, allowing therapeutic agents to cover all surfaces of the teeth [26]. While the flow test does not specifically measure the capacity to penetrate less exposed areas, it provides an indication of how the dentifrice responds to the pressure exerted by the toothbrush [27]. In the present study, GD demonstrated a higher flow than CG. This difference may be attributed to the absence of carrageenan, a thickening agent present in the control dentifrice. Increased flow ensures that ginger comes into contact with both the surfaces of the teeth and the oral mucosa during toothbrush.

To determine how the product interacts with tooth surfaces over time, the abrasion potential of dentifrices is an important aspect to consider. There are controversial estimates regarding the relationship between brushing test cycles and simulated brushing time. Some studies suggest that between 4,320 to 16,000 cycles simulate one year of brushing [28,29], while other research has considered up to 224,00 cycles to simulate 0.7 years of brushing [30]. Assuming an average of 25 to 30 cycles per day, with 10 cycles performed three times a day on each tooth surface [31,32] a total of 9,125 to 10,950 cycles can be estimated per year. Therefore, in the present study, 10,000 cycles were used to simulate one year of brushing with a soft toothbrush, in line with both previous studies and the current methodology [31,33,34]. Slurries were prepared by mixing dentifrice with water to simulate the dilution that occurs in the mouth due to saliva, which reduces the frictional action during brushing [34]. Some studies have used a dentifrice-to-water ratio of 1:1[31], while others have used a ratio of 1:3 [30], as was done in this study.

Surface roughness is a commonly used method for evaluating the abrasion potential of dentifrices, as comparing measurements before and after brushing provides insight into how the dentifrice affects the tooth surface over time [35]. The experimental dentifrice resulted in less change in enamel roughness along the y-axis, which corresponds to the brushing direction. The teeth in the GD group initially exhibited higher surface roughness, as a result of randomization to distribute samples between the two groups. Although the GD group initially showed higher surface roughness, the use of the control dentifrice led to an increase in roughness, and the final roughness did not differ between groups. The commercial dentifrice used as control contains silica, which serve as a reference standard for abrasiveness tests [36]. In contrast, the experimental dentifrice contains calcium carbonate ( $\text{CaCO}_3$ ), a common abrasive in commercial formulations [37,38]. Calcium carbonate has the potential to contribute to dental caries prevention, as it helps inhibit enamel demineralization and enhance remineralization [36]. With a Knoop hardness of 135 kg/mm<sup>2</sup>, calcium carbonate is softer than enamel (320 kg/mm<sup>2</sup>) [22]. However, some studies have reported that dentifrices containing  $\text{CaCO}_3$  may exhibit greater abrasiveness [39,40,41], possibly due to the particle shape. The composition of dentifrices, along with the characteristics of the abrasive particles such as hardness, shape, and distribution, can significantly affect their abrasiveness [42]. The combination of components in the experimental dentifrice resulted in less change to the enamel surface compared to the commercial dentifrice.

Higher surface roughness could lead to more pronounced color alterations [43]. No statistical difference was found between groups regarding the color parameters evaluated. Although ginger has a soft yellow coloration, it does not impact tooth color after brushing with a ginger-containing dentifrice, simulating one year of use. However, color changes with a  $\Delta E$  value of 3.7 or greater are considered clinically visible [24]. The commercial dentifrice, with a  $\Delta E$  of 4.84, resulted in a more noticeable color change compared to the experimental dentifrice ( $\Delta E$  of 3.87). This finding is consistent with the roughness analysis results, suggesting that the ginger-containing dentifrice may be less abrasive than the commercial dentifrice.

Laboratory studies are essential for understanding the performance and safety of materials, providing a solid foundation for the safe progression to in vivo testing. Future clinical studies are recommended to evaluate the antiemetic effects of the experimental dentifrice. Additionally, the anti-inflammatory and antimicrobial properties of the ginger-containing dentifrice could also be assessed.

## 5. CONCLUSION

The dentifrice containing ginger maintained cell viability and preserved the tooth enamel surface, with no significant changes observed in roughness or color parameters.

### COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.



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