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ORIGINAL ARTICLE

The Biocompatibility of a Ginger-Containing Herbal Toothpaste on Developing Zebrafish Embryos

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ABSTRACT

The biocompatibility of toothpaste in an oral cavity should be approved by clinical trials. Nowadays, herbal toothpaste is increasing in popularity due to its natural ingredients. Being genetically similar to humans, zebrafish are used in potential toxicity testing. The zebrafish embryotoxicity test is a fast and straightforward method to study chemical toxicity during embryogenesis. **Objective:** This study aimed to evaluate if there was any biocompatibility of the toothpaste on zebrafish embryos. **Methods:** Adult AB strain zebrafish were used according to Institutional Animal Care and Use Committee protocols. Normally dividing, spherical embryos were exposed to herbal toothpaste with ginger (Gumgumix, Turkey) (50 mg/L) and conventional toothpaste (Signal, Expert Protection, Bulgaria) (50 mg/L) in well plates containing 20 embryos, having four replicates. Developmental effects, mortality, and hatching rates were evaluated for 72h. **Results:** Zebrafish embryos exposed to conventional toothpaste had a higher mortality rate than those exposed to herbal toothpaste; they hatched later and delayed in development. There was no difference between herbal toothpaste and the control group regarding mortality and hatching rates ($p > 0.005$). **Conclusion:** The herbal toothpaste showed higher biocompatibility on zebrafish embryos compared to the conventional toothpaste under the condition of this study.

Key words: development, embryo, toothpaste, toxicity, zebrafish

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INTRODUCTION

Tooth brushing, the basis of oral care, provides mechanical cleaning. Toothpaste is used to aid tooth brushing and is an indispensable product for personal oral hygiene. Toothpaste was used as a tooth cream consisting of pumice and ash between 3000-5000 BC. With the addition of glycerin, it became what we now know as a toothpaste form. Various chemical substances were added to the chemical content of toothpaste in time.[1] Detergent, or surfactant, is added to toothpaste to provide remineralization and antibacterial effects due to its hydrophilic and hydrophobic properties.[2] The most common detergent used in toothpaste is the anionic compound sodium lauryl sulfate (SLS, $C_{12}H_{25}NaO_4S$), which is at a concentration of 0.5-2% by weight in toothpaste.[3]

Many fluoride compounds are used in the structure of toothpaste to prevent dental caries. With the addition of fluoride in their contents, toothpaste gained caries-preventing and remineralization properties. Since then, fluoride compounds such as sodium fluoride, sodium mono fluoro phosphate, amine fluoride, acidulated phosphate fluoride, and stannous fluoride have been added to toothpaste.[4] The effect of fluoride on enamel is positive when taken in optimal doses. While long-term fluoride intake at optimal doses is not harmful to the human body, "acute fluoride toxicity" occurs with fluoride intake at one time and high doses, and "chronic fluoride toxicity" occurs with fluoride intake over the optimal dose and for a long time.[5]

The tendency to be "natural" has led to an increased demand for herbal products by consumers. Herbal products in dentistry are limited compared to synthetic drugs in other biomedical fields. Many oral care product manufacturers have added herbal ingredients to their products. While the conventional toothpaste formula is preserved in these products, herbal extracts are added to their ingredients. However, the fact that they contain plant extracts does not mean these products constitute herbal toothpaste. A large percentage of their content still consists of synthetic additives and preservatives. In some products, manufacturers have enriched their formulations with herbal extracts by removing chemicals thought dangerous, such as fluoride, SLS, triclosan, and parabens. Some products have removed some of these chemicals (such as fluoride and/or SLS-free or paraben-free toothpaste), and herbal products with active ingredients are mostly plant-based. Formulations containing plant extracts and natural ingredients are in the form of toothpaste, mouthwashes, and gels. The carbohydrates, amino acids, fatty acids, minerals, vitamins, enzymes, and phytochemicals they contain provide the effectiveness of these natural substances. These herbal extracts and natural ingredients include aloe vera, chamomile, ginger, green tea, thyme, turmeric, miswak, peppermint, lavender, tea tree oil, and sage.[6] An herbal renaissance period has almost started worldwide and with increasing importance. Among the reasons these natural products are preferred instead of synthetic products are that these products are not tested on animals, do not use animal products, do not have side effects, and are vegan-friendly. In addition, toothpaste labeled as "natural" typically does not contain synthetic sweeteners, artificial colors, preservatives, additives, or synthetic flavors and fragrances.[7] Despite the possible toxicity effects of chemical substances in toothpaste, biocompatible herbal toothpaste that will not cause these effects is in demand.[8] However, there are few studies on these products in the literature.

Due to the chemical content of toothpaste, biocompatibility also needs to be searched. Among the model organisms used for the toxicological evaluation of chemicals and drugs, zebrafish are also accepted as the "gold standard" recently.[9] Zebrafish is a 4-6 cm long freshwater fish, has 84% of human disease genes, and can breed many embryos simultaneously with external fertilization. Externally fertilized zebrafish embryos grow and develop quickly. Both embryos and larvae are transparent, making them accessible for observation and manipulation in all development stages. So, they are considered more advantageous than common mammalian organism models (for example, mice). The Zebrafish embryo toxicity test is a fast and simple method to study chemical toxicity during embryogenesis.[10]

The hypothesis of this study was determined as "Herbal toothpaste is biocompatible due to their natural content." The aim of this study was to evaluate the effects of a ginger-containing herbal and a conventional toothpaste on developing zebrafish embryos.

Table 1. Toothpaste's ingredients

Toothpaste	Ingredients
Signal Expert Protection (Unilever, Bulgaria)	Sodium Monofluorophosphate, Silica, Potassium Citrate, Zinc Citrate, Hydroxyapatite, PEG-32, Sodium Lauryl Sulfate, Trisodium Phosphate, Cellulose Gum, Sodium Hydroxide, Sodium Saccharin, Cl 74160, Cl 77891
Gumgumix (Beka Drug, Turkey)	Calcium Carbonate, Glycerin, Water, Honey, Licorice Root, DiCalcium Phosphate, Ginger Extract, Xanthan Gum, Sodium, Carboxymethyl, Potassium Sorbate, Menthol, Sodium Benzoate

METHODS

The research was conducted in the Marmara University Basic Medical Sciences Department. Zebrafish embryos were used for the research at 72 hours post fertilization (hpf). As the zebrafish embryos used were no older than five days old, no ethical approval was required for the protocols applied as stated by the Council of Europe (1986), Directive 86/609/EEC. Male and female (2:1) zebrafish were kept in an aquarium rack system at 28°C under a 14/10 light/dark cycle (Figure 1). Fertilized eggs were collected, and embryos were rinsed under water several times before their use. Normally dividing, spherical embryos were exposed to a conventional toothpaste (50 mg/L) (Signal Expert Protection, Unilever, Bulgaria) and an herbal toothpaste (50 mg/L) (Gumgumix, Beka Drug, Turkey) solutions (Table 1). E3 medium solution was placed in well plates for the control group. E3 medium contains 5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, and 0.33 mM $MgSO_4$ for 1 L. The toothpaste solutions were prepared in E3 (50 mg/L). In total, 240 embryos were used. Four independent experiments were conducted. Accordingly, each group was prepared as four replicates in well plates, with 20 embryos in each. They were monitored to evaluate their development, and the images of malformations were recorded using a stereomicroscope (Zeiss Discovery.V8). Mortality and hatching analyses were also conducted every 24 hours. The mortality and hatching rates were analyzed blindly. The hatching rate is the ratio of hatched embryos to the number of live embryos in each well. Each day all the exposure solutions used in the study were changed and replaced with fresh solutions. At the end of 72 hpf, the exposure period ended, and the zebrafish embryos were washed several times using water (Figure 1).

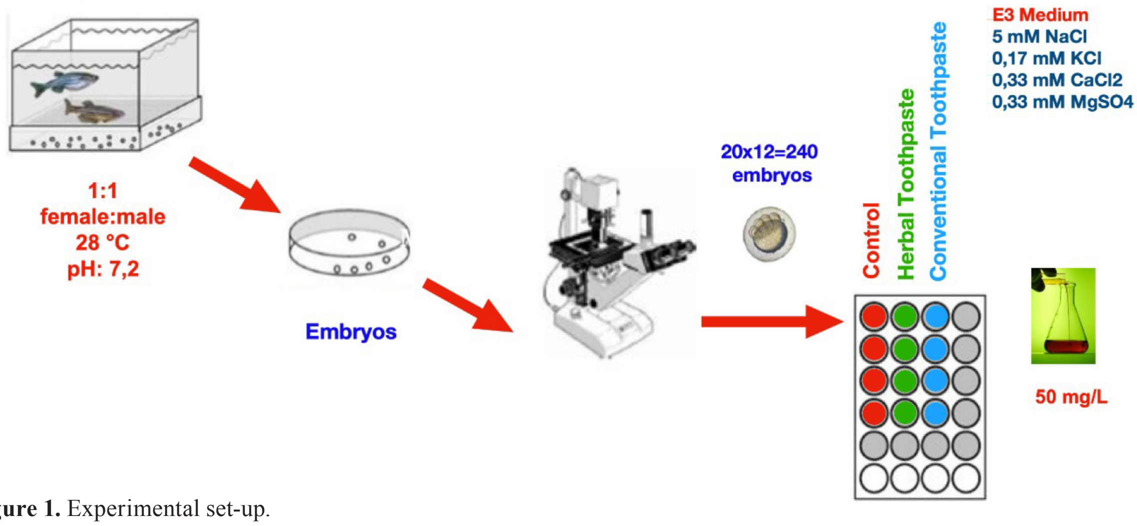

Figure 1. Experimental set-up.

Figure 2. Representative images of zebrafish embryos taken by stereomicroscobe at 72 hpf. Embryos exposed to the herbal toothpaste (Gumgumix) developed morphologically similar to the control group. Yolc sac edema was observed in conventional toothpaste exposed group. * Indicate yolc sac edema.

Statistical analysis

GraphPad 9 was used to evaluate the differences between conventional and herbal toothpaste groups and control groups. First one-way analysis of variance (ANOVA) analysis was applied, followed by Tukey's multiple comparison tests to evaluate the differences between the groups. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Zebrafish embryos were evaluated for developmental parameters under a light microscope (Zeiss Discovery, V8) at the end of 72 hpfs. The development parameters for embryo staging included the yolk sac, anal pore, pectoral fin, and swim bladder. The representative images of the zebrafish embryos at 72 hpf are given in Figure 2. Embryos exposed to herbal toothpaste developed morphologically similar to the control group. Yolc sac edema was observed in the conventional toothpaste group (Figure 2).

The mortality rate of zebrafish embryos at 72 hpf was compared with One Way ANOVA followed by the Tukey Multiple comparison test. Conventional toothpaste-exposed zebrafish embryos had an increased mortality rate which was significantly higher than the group exposed to the herbal toothpaste and the control group ($p < 0.05$). There was no significant difference between the group exposed to herbal toothpaste and the control group ($p > 0.05$) (Figure 3).

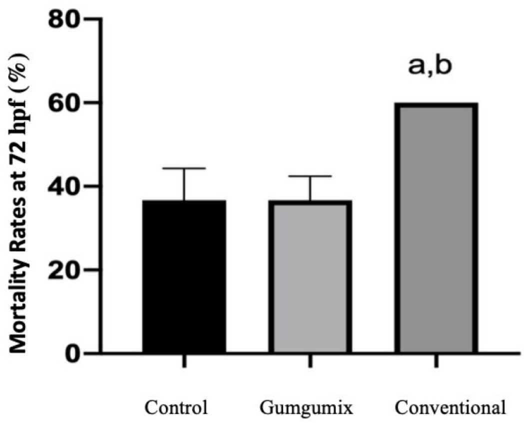

Figure 3. Mortality rates of the groups. Values represent means ± SD. Significant differences between the groups were analyzed by ANOVA test followed by Tukey's multiple comparison test. [a,b] indicate significant differences between groups ($p < 0.05$), [a] $p < 0.05$ significantly different than the control group; [b] $p < 0.05$, significantly different than Gumgumix group.

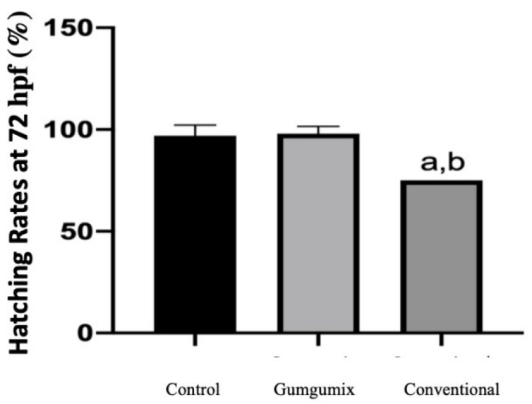

Figure 4. Hatching rates of the groups. Significant differences between the groups were analyzed by ANOVA test followed by Tukey's multiple comparison test. Values represent means ± SD. [a,b] indicate significant differences between groups ($p < 0.05$), [a] $p < 0.05$ significantly different than the control group; [b] $p < 0.05$, significantly different than Gumgumix group.

The hatching rate of zebrafish embryos at 72 hpf was compared with the One Way ANOVA followed by the Tukey Multiple comparison test. Accordingly, the hatching rate of the conventional toothpaste group was significantly lower than the herbal and control groups ($p < 0.05$). There was no significant difference between the group exposed to herbal toothpaste and the control group ($p < 0.05$) (Figure 4).

DISCUSSION

Our study is the first to evaluate toothpaste (as a whole product) since there is no other study in the literature evaluating any effect of toothpaste during the development of zebrafish embryos.

In a limited number of studies with zebrafish embryos, Bartlett et al. established fluorosis on the teeth of zebrafish, which have a hard enameloid surface. This was shown by a significant increase in organic content on pitted and rough surfaces.[11] Zhang et al. applied different doses of fluoride to zebrafish larvae for five days to induce dental fluorosis.[12] It was reported that there was a significant decrease in DNA, RNA, and protein levels in the animal's brain, cerebellum, and medulla oblongata regions, depending on the increased fluoride level.[13] Two studies on fluoride toxicity stated that fluoride causes genotoxicity.[5,14] In our study, the probable biocompatibility of the conventional toothpaste with 1450 ppm fluoride was lower than the herbal toothpaste without fluoride. The mortality and hatching rates were the main criteria for the decision of toxicity of different types of toothpaste in the present study. Fluoride contents of conventional toothpaste might increase the toxicity effect due to the bacteriostatic effect of fluoride acting synergically with SLS.[15]

Another toothpaste ingredient related to toxicity is SLS. Oliveira et al., one of those rare studies, found that triclosan causes acute toxicity in zebrafish embryos reducing hatching rate, pigmentation, and stature.[16] Although triclosan was added to the toothpastes to benefit from its antibacterial effect, in Olivieria's study, it was reported to have a negative effect on the hatching rate. In the present study, the conventional toothpaste (Signal) caused a lower hatching rate similar to Oliveira et al.'s study, besides an increase in mortality rate. This might be possible due to sodium lauryl sulfate, which uses antimicrobial effects in toothpaste. In addition to its beneficial antimicrobial effects, SLS is thought to have adverse effects on patients with recurrent aphthous ulcers. SLS causes desquamation in the oral mucosa. SLS-induced elimination of the protective mucin surface layer reduces the resistance of the oral mucosa.[17,18] It has been shown that there is a relationship between increased oral desquamation and toothpaste use.[19-21] In the study by Shim et al., it was stated that the duration of the ulcer and the pain score decreased significantly when SLS-free toothpaste was used, compared to the period in which SLS-containing toothpaste was used.[22] Ulceration and inflammation caused by SLS have been shown in different studies.[21,23,24] SLS also causes marked histological changes in the buccal epithelium. These changes cause hyperkeratosis, acanthosis, basal cell hyperplasia, and increased epithelial thickness.[25] In an in vivo study conducted by Tadin et al. in 2019, examining the effects of SLS on human buccal epithelial cells, it was found that SLS changed the nuclear morphology in buccal epithelial cells, which in turn increased epithelial thickness.[26]

Our research in 2020 showed no significant difference in toxicity between low doses of SLS and the control group in the development of zebrafish embryos. However, the mortality rate was significantly higher than the control group.[15]

Various studies have investigated the biological effects of sodium benzoate, another antimicrobial additive, on cell and animal models. It was reported to suppress the cellular immune response at non-toxic concentrations.[27] It has been determined that it causes cytotoxicity and genotoxicity in lymphocytes.[28] In the cytotoxicity study conducted by Gaur et al. on zebrafish larvae in 2018, 100% mortality was observed in the larvae at concentrations of 500 ppm and above at 72 hours. At concentrations above 200 ppm, the mortality rate was significantly higher than the control group.[29] The reason that sodium benzoate as an ingredient in the herbal toothpaste was found biocompatible might be due to the lower dose used as limited by the health ministry of our country.

CONCLUSION

Our research shows that the biocompatibility of ginger-containing herbal toothpaste on zebrafish embryos is higher than the conventional toothpaste with SLS and fluoride in the study. Toothpaste has many benefits, including caries prevention, remineralization, and whitening. However, many toothpaste ingredients can enter systemic circulation and be stored in various organs. For this reason, especially for children, toothpaste suitable for the age group should be used, and both children and adults should be careful not to swallow any toothpaste. Although the concentration of the ingredients added to the toothpaste is minimal, caution should be taken due to the danger of toxicity because the same ingredients may be ingested through food, water, and other personal care products.

CONFLICT OF INTEREST

All authors declared no conflict of interest.

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